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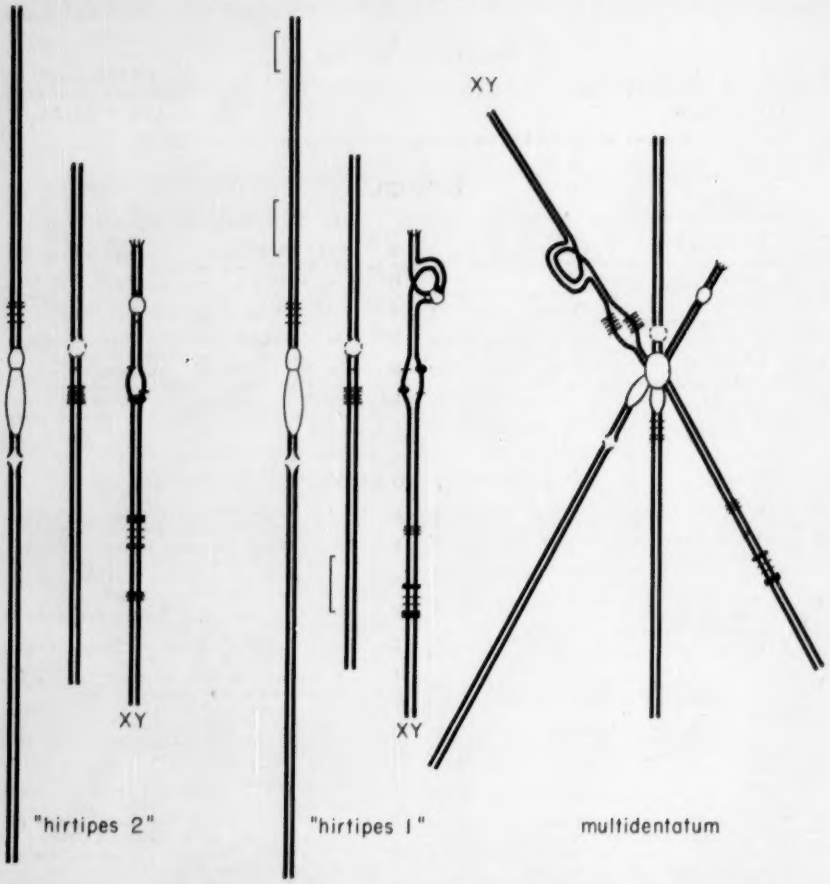

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STEEL MICE DWARF CATTLE
WOOLLY CHICKENS AND HYBRID TURKEYS
DROSOPHILA INTERCHROMOSOMAL CROSSOVERS

Volume 47 MAY-JUNE, 1956 Number 3

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DWARF HEREFORD-ANGUS HYBRIDS

Figure 1

A and *B*—Photographs of dwarf females, 82 and 83, at approximately 23 months of age, and *C*—dwarf male 133, at approximately 12 months of age. All were produced from the mating of normal registered Aberdeen-Angus bulls to normal high grade Hereford cows. All of these dwarfs, manifest the characteristics of F_1 Hereford-Angus hybrids in that all have black coats with white facial markings. They also possess typical characteristics of short-headed dwarf segregates that occur in the parent herds of registered Aberdeen-Angus and registered Herefords.

EVIDENCE FOR THE SAME DWARF GENE IN HEREFORD, ABERDEEN-ANGUS, AND CERTAIN OTHER BREEDS OF CATTLE

P. W. Gregory and F. D. Carroll*

On the basis of phenotypical appearance alone, it has been assumed that the type of dwarf found in Herefords and studied by Craft and Orr,[2] Johnson *et al.*,[10] Gregory and co-workers,[4,5,6] Lindley,[14] Carroll *et al.*,[1] and Lush and Hazel[15] was identical with one of similar morphological form commonly encountered in the Aberdeen-Angus breed. Reports supporting the hypothesis that the dwarf gene is common to both Hereford and Aberdeen-Angus breeds have reached the authors from time to time. These reports have come from cattlemen who have observed sporadic dwarfs that were produced from the mating of Hereford cows to Aberdeen-Angus bulls in the production of commercial cattle. However, there has been no evidence reported in the literature to support the hypothesis that this dwarf conditioning gene is common to both breeds. All contacts with breeders and commercial cattlemen clearly indicate that the incidence of dwarfism in the major beef breeds is increasing. Furthermore, matings under controlled conditions indicate that the dwarf problem is more complicated than the original field studies and surveys have indicated.

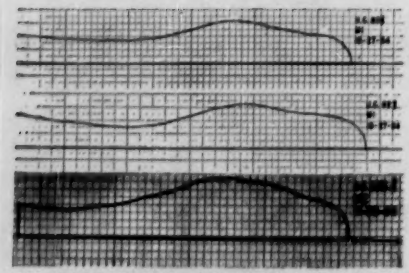
There are many different phenotypical forms of dwarfism, and there are good reasons for assuming that several different genes are involved in conditioning most of the diverse dwarf phenotypes.[7,8,13] Perhaps the most distinctive feature of the type of dwarf discussed in this paper is a premature closure of the spheno-occipital synchondrosis.[11] If order is to be established with respect to the inheritance of bovine dwarfism, the genetic relationships of the phenotypically different dwarf forms must be determined.

Since factual information is meager or lacking on all the dwarf types, the authors feel that any data which definitely settles a single point should be recorded promptly to make the research of all investigators more effective. The purpose of this report is to present evidence from critical mating tests made under field conditions which clearly indicates that the recessive dwarf gene which conditions the short-headed type of dwarf is common to both Hereford and Aberdeen-Angus breeds. Evidence of another type will be presented which indicates that this gene is also present in other breeds.

Source of Data and Analysis

The test progeny providing the critical evidence were produced in two commercial herds in California that were mating high grade Hereford cows to Aberdeen-Angus sires. One herd was in the selective breeding program of the California Agriculture Extension Service and the cows and sires used were above average grade for commercial herds. The other herd was not in the program. All the Aber-

*University of California, Davis, California. This study was supported by the Kellogg Fund of the University of California for the Application of Genetics to Farm Mammals, and was conducted under California Experiment Station Project 1451 in cooperation with the U.S.D.A. and the State Experiment Stations under Western Regional Project W-1 which concerns Beef Cattle Breeding Research. The authors express with pleasure their indebtedness to several members of the California Agricultural Extension Service for bringing to their attention the dwarfs and dwarf hybrids used in this study. Mr. H. S. Strong, Extension Specialist, and Mr. Albert E. Weber, a former farm advisor of Los Angeles County, discovered and made the necessary arrangements which put five dwarfs from one herd at our disposal. Mr. Don A. Petersen, farm advisor of Merced County, observed the hybrid dwarf bull and made the arrangements that allowed him to be used in the study. Mr. Roy V. Parker located the Shorthorn dwarf heifer and provided all the first-hand observations and photographs. The authors are also indebted to all the cattlemen who cooperated on the study and donated dwarfs for experimental use.



HEAD PROFILES OF THREE DWARFS

Figure 2

Median head profiles of dwarf females 82 and 83 taken at approximately 24 months of age and dwarf male 133 taken at 12 months of age. These profiles are typical of Hereford dwarfs of this age and sex.

deen-Angus sires used were registered.

In the first herd there appeared in the 1952-1953 calf crop five F_1 hybrid calves from the crossing of Hereford cows to an Aberdeen-Angus sire that were definitely and unmistakably identified as dwarfs. This was a small mating group and included all the Hereford replacement heifers which were placed in one pasture with one Aberdeen-Angus bull. This cattleman kept good records and there was reasonably accurate classification of each of the progeny. There are uncertainties as to the exact number of replacement heifers used in the mating group, but it is approximately 28. There is also some uncertainty as to the absolute number of total crossbred progeny that were produced. However, there is absolute proof that five F_1 hybrid Hereford-Aberdeen-Angus dwarf calves were produced. All five dwarfs were donated to the University of California and placed in the California Agricultural Experiment Station dwarf herd for special studies. They were brought to Davis in the spring of 1953 at about five months of age and were under close observation thereafter. Three died from bloat within a few weeks after they arrived at our laboratory. The two that survived were females (Figure 1*A* and *B*). Their exact date of birth is unknown, but the approximate date is November 1, 1952. It has been mentioned that there are slight uncertainties as to the number of heifers used in the mating and the absolute number of normal calves produced. Furthermore, there is no critical classification with respect to dwarfism of calves aborted late in pregnancy, stillbirths, or calves that died early. This is important since there are strong indications that dwarf calves manifest a higher post-natal mortality than normal calves, and it is also known that it is difficult to recognize some dwarfs at a young age. It is highly significant that the five dwarf calves are positively and accurately identified. Since this type of dwarfism is conditioned by an autosomal recessive gene the sum total of any or all errors that may have been made cannot be great.

The appearance of these hybrid dwarfs closely resembles the dwarfs commonly observed in Herefords, as is evident when Figure 1 of this report is compared with the illustrations of Craft and Orr,[2] Lindley,[14] and Johnson, *et al.*[10] Furthermore, the general phenotype of these hybrids and their respective head profiles, shown in Figure 2, are identical in appearance with the animals studied by Gregory and co-workers.[4,5,6] The occlusion of the incisor teeth on the dental pad of these hybrid dwarfs is similar to Hereford and Aberdeen-Angus dwarf females of the same phenotype as illustrated by Gregory *et al.*[4]

Definite evidence of other similar F_1 dwarf hybrids produced under commercial operations has been brought to the attention of the authors. The dwarf bull calf shown in Figure 1*C* was produced in a commercial herd that was using grade Hereford cows mated to registered Hereford and Aberdeen-Angus bulls. Even if one were unaware of the mating practice employed upon this ranch, the black color, white facial pattern, and polled condition give ample evidence that this crossbred dwarf arose from parents of Hereford and Aberdeen-Angus breeding. Two additional dwarf hybrid calves involving these two breeds have come to our attention. Both had black coats with white faces. There can be no doubt of their authenticity.

These six F_1 hybrid dwarfs studied in detail and the two others mentioned from the crossing of Aberdeen-Angus bulls of normal size on high grade Hereford cows of normal size are of a phenotype similar to dwarfs that commonly segregate out from parents of normal size, in both parental breeds. The dwarf type in Herefords is definitely conditioned by an autosomal recessive gene.[15,6] As yet no genetic analysis has been made of this type of dwarfism in the Aberdeen-Angus breed, but the pedigrees in the hands of the authors clearly indicate that the dwarf in this breed is also conditioned by an autosomal recessive gene.

It should not be assumed, however, that all the relationships concerning the expression of recessive dwarfism in crosses between Hereford and Aberdeen-Angus breeds are solved. Since the F_1 hybrid dwarfs were not mated to produce an F_2 population, it may be argued that two or more dwarf conditioned genes with unique factor interaction may be involved in producing these dwarfs. There is no evidence in either breed of the existence of a dominant gene that might condition dwarfism. Thus with the data at hand, the conclusion that the same recessive dwarf gene is common to both Hereford and Aberdeen-Angus breeds is the simplest interpretation.

Many dwarfs are grossly deformed and suffer from lack of vigor. The two hybrid dwarf

A SHORTHORN DWARF
Figure 3

Photographs showing two views of a Shorthorn dwarf heifer two years of age. Note the extreme short legs, pot belly, and the long lower jaw that extends beyond the upper jaw. Some idea of the degree of dwarfing may be obtained by comparing the dwarf with the normal two-year old heifer that is partially shown.

heifers, Nos. 82 and 83 (Figure 1), are typical dwarfs, yet they do not appear extremely misshapen and manifest more than average vigor and good health. Since they are two years of age, it is of interest to compare some of their specific body measurements with the mean body measurements of 52 normal Hereford females two years of age reported by Guilbert and Gregory.[9] With perhaps the exception of the round measurement discussed in detail by Gregory[3] and Kidwell *et al.*,[12] all the body measurements are well known or are self-explanatory.

These normal females are from the University of California Experiment Station herd. Although there could be a few that are heterozygous for recessive dwarfism, by far the greater percentage should be homozygous normal. The body weight of these dwarfs is half that of normals, while most of the linear measurements range from approximately two-thirds to three-fourths of the value of the normals. It may be observed that the head width, cannon bone width, and round measurement are 96 percent, 90 percent, and 106 percent of normal respectively. These characteristics are assumed to be of biological significance and may have a specific bearing on the selection of breeding stock.

Discussion

Whether or not this type of dwarf produced from breed crossing benefits from hybrid vigor

is an interesting question. Certainly, Nos. 82 and 83 are above average in vigor. On the other hand, the hybrid dwarf bull may be less vigorous if harder breathing, a greater tendency to bloat, and general appearance are valid criteria of heterosis. This, coupled with the fact that three of the dwarf hybrids from herd 1 died when brought to our laboratory, makes it extremely doubtful that hybrid vigor, *per se*, favorably affects the dwarfs produced from breed crossing.

The distribution of the recessive dwarf gene in other breeds of *Bos taurus* is of both theoretical and practical interest. We have observed from time to time dwarfs of Shorthorn breeding that are morphologically similar to those shown in Figure 1. The dwarf Shorthorn heifer two years of age shown in Figure 3 manifests a bulging forehead, broadness between the eyes, disproportion between the upper and the lower jaw resulting in malocclusion, marked pot belly, and labored breathing typical of short-headed dwarfs of Hereford and Angus breeds. All of these characteristics show such detailed phenotypic analogy to the short-headed dwarfs of the Hereford and Angus breeds that genetic homology is strongly suspected.

Reports which have strong indications of authenticity have reached the authors that dwarfs have been observed in the Holstein and other dairy breeds. Evidence based upon critical mating tests will have to determine whether the Shorthorn and the possible dwarf mutants in the dairy breeds are identical with those of the Hereford and Aberdeen-Angus breeds.

It is obvious that there are many dwarf mutants in breeds of beef cattle as well as in dairy breeds. It is of biological importance and may be of practical signficance whether these mutations are of recent origin or occurred some time ago and have been carried indefinitely in an unfixed condition in the several breeds. Testimony of older veterinarians, cattlemen and breeders indicates that dwarf segregates similar to those now being observed were occurring in cattle of Hereford and Shorthorn breeds fifty years ago or more. Thus the dwarf mutation is not regarded as of recent origin. The stability of a locus is relative rather than absolute and the mutation may be reversible. Furthermore, it must be assumed on theoretical grounds that essentially the same potentiality for mutation (locus change) exists in all the breeds. What breeders and cattlemen should realize is that once a specific mutation has been found in a breed (or species) this mutation is potentially present in all breeds or sub-divisions of the species. When all things are considered, perhaps the most tenable hypothesis is that the dwarfs that are segregating in the cattle breeds are not the product of recent mutations but of mutations that occurred many generations ago, perhaps before the breeds were established. If this is true, once the mutation has occurred, selection pressure should have a greater influence on gene frequency than mutation rate.

TABLE I. Data from two crossbred dwarfs and the average of 52 normal Hereford heifers

	Dwarf 82	Dwarf 83	Herefords	Average 2 dwarfs / Average Herefords × 100
Weight*	505	510	1006	50.4
Height of withers†	91	91	114	79.8
Height at hooks	92	93	119	77.7
Heart girth	140	141	180	78.0
Body length	107	108	142	74.1
Round measurement	91	88	105	85.2
Width of head	21	21	21.9	95.9
Length of Head	16.2	15.5	44.6	80.4
Width of hooks	34	33	49.4	67.8
Diameter of cannon bone Round	4.4	4.6	5.0	90.0
Height Withers / Head Length	1.00	0.97	.92	106.6
Head Width	1.73	1.69	2.04	83.8

*In pounds.
†All linear measurements are in centimeters.

Summary

Data collected from the herds of California commercial cattlemen who are using registered Aberdeen-Angus bulls on high-grade Hereford cows clearly indicate that crossbred (F_1) dwarf hybrids are produced which are similar in all diagnostic features to the dwarfs that normally segregate in both parental breeds. In the absence of genetic tests of the crossbred (F_1) dwarfs several genetic hypotheses may be advanced to explain these results. The simplest and the most tenable one, however, is that both Hereford and Aberdeen-Angus breeds carry the same recessive dwarf gene. Evidence based upon morphological characteristics alone indicates that this type of dwarf also occurs in the Shorthorn and Holstein breeds. Comparisons of some body measurements and body proportions of two crossbred (hybrid) dwarf heifers two years of age, produced from the crossing of normal Hereford and normal Aberdeen-Angus parental stock, are made with normal Hereford heifers of the same age.

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◆

THE FREQUENCY OF MULTIPLE BIRTHS IN PAVIA IN THE 16TH AND 17TH CENTURY

M. Fraccaro*

A MOST interesting study of the demographic and economic aspects of the city of Pavia during the 16th and 17th century has been made by G. Aleati[1] of the "Ufficio Statistico del Comune" of Pavia (Lombardia, Italy). We owe the present data to his courtesy. Human population genetics is now changing from a mostly theoretical phase and collections of actual data have been initiated in several countries, *e.g.* in France by Sutter and Tabah,[8] in Italy by Cavalli Sforza[4] and in Sweden by Böök. [2,3] The need for such data was also emphasized by a number of human geneticists at the 1954 World Population Conference.[7]

Studies of the dynamics of human populations must frequently be based on "historical data" such as old parish books and *libri familiae*. This presents the research worker with problems of great difficulty as far as the reliability and the interpretation of old registers are concerned. We do not intend to discuss such problems here and for details regarding the registration of the present data the reader is referred to the book of Aleati,[1] where a full account is given.

The data presented here have mostly the character of a curiosity, as other data than twin births are much more important for population genetics, *e.g.* cousin marriages, differential fertility. In Table I the total number of births (*i.e.* the number of newborns *baptized*) in 17 parishes of the city of Pavia is given together with the years in which they were registered. The decision whether to baptize a child depended mostly on its being 'alive,' so that most of the stillbirths have not been included. At the same time the priest was generally doing his best to try to save as many souls as possible and this was responsible for a wide range of variation of the concept of 'living soul.' For discussion and amusing anecdotes see Aleati's book. The same table shows the sex combi-

*Assistant Director, the State Institute for Human Genetics, Uppsala, Sweden.

nation within the twin pairs, *i.e.* like-sexed male pairs (MM), like-sexed female pairs (FF) and mixed pairs (MF), the totals and the frequency of twins as a percentage of all births. A few triplets have been recorded (Table II) while no quadruplets or other multiple births were disclosed. The frequency of twin births is about 1:150 (cf. in Pavia 1:72 in 1955; in Italy 1:80 in 1949-1950).[6] Even if high incidence of stillbirths among twins can be anticipated for this period one reason for this low figure is probably that when one partner of a twin pair was stillborn, the surviving partner was not entered as a twin in the register. The heterogeneity of the data is also pronounced (χ^2 for homogeneity=46.5 for 16 D.F.) but no satisfactory explanation can be given as the differences between parishes may be due to *inter* group biological or social differences as well as simply to varying practices of registration.

The incidence of triplets (1:10,308) is higher than expected as compared to the frequency of twins, *i.e.* 1/150[2] or 1:22,500.

The distribution of the pairs according to sex combinations is in agreement with contemporary twin statistics. From the 93 MM, 94 MF and 89 FF pairs we can calculate that 45 of the MM and 43 of the FF pairs or 32 percent of the twins should be monozygotic. A sample of twin births collected by the author[5] in a Maternity Clinic in Pavia showed the proportion of 84 MM, 78 MF and 75 FF, of which 42 of the MM and 36 of the FF pairs or about 34 percent should be monozygotic.

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TABLE II. Triplet births

Parish	Year	Triplets
S. Maria Nova	1660	M F F
S. Giovanni Domnarum	1635	M F F
S. Primo e Feliciano	1692	M F F
S. Giovanni in Borgo	1588	M M M

TABLE I. Total numbers of births in 17 parishes of Pavia in the 16th and 17th Century

Parish	Years	All births	Twin births			Total twin births	Relative frequency of twin births, %
			MM	MF	FF		
S. Invenzio	1559-1700	1747	9	4	5	18	1.03
S. Pantaleone	1578-1700	560	–	1	2	3	.54
S. Trinitá	1563-1700	2748	9	9	5	23	.84
S. Patrizio[1]	1577-1700	1913	7	12	5	24	1.25
S. Maria Nova	1578-1694	2468	1	4	10	15	.61
S. Maria Capella	1565-1700	3354	8	8	11	27	.81
S. Andrea in Cittadella[2]	1564-1686	798	2	2	1	5	.63
S. Giovanni Domnarum	1566-1700	3647	4	9	7	20	.55
S. Maria Canonica Gualtieri[3]	1581-1700	3301	7	5	4	16	.48
S. Romano	1570-1700	1763	1	2	1	4	.23
S. Primo e Feliciano	1574-1700	3044	7	5	6	18	.59
S. Giovanni in Borgo[4]	1564-1700	4389	17	20	10	47	1.07
S. Maria Costa Cremona[5]	1567-1700	2667	7	6	5	18	.67
S. Maria Perone[6]	1576-1700	2186	2	—	—	2	.09
S. Francesco	1564-1656	1894	4	3	5	12	.63
S. Marino	1565-1700	2533	4	3	6	13	.51
S. Eusebio	1576-1697	2222	4	1	6	11	.50
Total		41234	93	94	89	276	.67

1. No records for the years 1655-1685
2. No records for the years 1574-1575 and 1593-1605
3. No records for the years 1629-1630
4. No records for the years 1590-1597
5. No records for the years 1614
6. No records for the years 1637-1641

BLACK FLIES: SIBLINGS, SEX, AND SPECIES GROUPING

K. H. ROTHFELS*

THE purpose of this paper is to illustrate with one or two examples certain analytical methods which we have used in a comparative cytological study of black flies, and to draw to the attention of others the enormous possibilities inherent in such an approach to the group.

The somatic complements as seen in ganglion smears, are surprisingly uniform, and in approximately 35 species conform to that described and figured for *Simulium vittatum*.[5] The three metacentric chromosomes of the typical complement have been numbered I, II, III, in decreasing order of size; short arms are symbolized by *"s"* and long arms by *"l"*. Exceptional species which may be recognized from somatic complements include three triploids (*Gymnopais* spec., *Prosimulim "0"*, 3n "form" of *Cnephia mutata*), *Prosimulium decemarticulatum* with an extreme arm ratio in III, and *Eusimulium aureum* and siblings with a haploid complement of two.

Again, the study of meiosis in the male is not likely to furnish specific diagnostics. Meiotic parameters of all species investigated so far approximate those described by Montalenti[4] for *Simulium equinum*. Chiasma frequency per arm is low, terminalization moderate, and interference across the centromere strong. The triploids will prove the one certain exception to this uniformity; a study of their reproduction is just beginning.

Study of the salivary gland chromosomes immediately reveals a tremendous variation in gross features of complements and in details of banding, with rather shattering implications as far as the uniformity of the mitotic complements is concerned. The salivary gland chromosomes of *S. vittatum* were described in detail[5] (see also for earlier literature) and indications were given of the usefulness in species diagnosis of certain gross characteristics including: number of chromosomes, degree of ploidy, qualitative features of centromere regions, position of nucleolus and the so-called Ring of Balbiani, degree of pairing, and arm length in percent total complement length (percent TCL). To this list may be added the occurrence, in a few species, of a chromocenter, first described by Kunze[2,3] in an unnamed Austrian species, which from its chromosomes appears to be a *Eusimulium* close to the North American *pugetense* (*costatum*?). Distinctive chromocenters also occur in certain nearctic *Prosimulium* and *Eusimulium*. Where chromocenters are absent the exact position of the centromere within the central heterochromatic structures can in most species be determined by the frequent occurrence of ectopic pairing. Centromeres so identified conform in position to those of somatic chromosomes and to the regions involved in chromocentric fusions in related species. Ectopic pairing of interstitial segments is rare in black flies and, where analyzed, attributable to short euchromatic duplications.

Cytological diagnoses are much refined if, in addition to gross features, details of the banding pattern are invoked. These are particularly clear in most species. Moreover, in contrast to Drosophilids, black flies are sufficiently conservative in their evolution to allow reliable interspecific or even intergeneric comparisons of the banding pattern without recourse to hybridization. Thus species may be identified not only from maps of significant parts of their complement but also from explicit descriptions of the fixed rearrangements which

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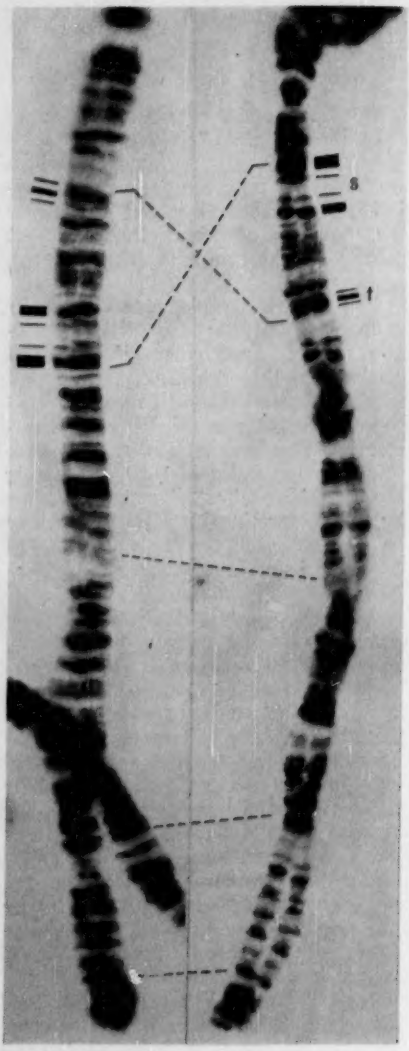
separate them from their known relatives. Further characterization is possible by a determination of the highly individualistic spectra of floating rearrangements, notably inversions which are common in most species.

It is perhaps not unexpected that the application of these micromorphological methods reveals the existence of biologically distinct species (siblings) which have not been differentiated by the conventional taxonomic approach. More surprising is the discovery of the widespread occurrence of a distinctive sex determining mechanism of the XY type with male heterogamety and employing inversions in the evolution of differential segments. Finally, the studies show that rearrangements may be exploited as phylogenetic tracers to group species according to their evolutionary affinities in terms of common ancestors.

Sibling Species in *Prosimulium hirtipes*

Figure 4 shows the III *l* ends representative of two classes of individuals which on the basis of larval, pupal and adult characters would key out as *P. hirtipes*. Homologous bands are joined by broken lines. The terminal homology extends at least as far as the guide line in the center of the figure. Two further direct homologies between these chromosomes may be understood from Figure 5. The first comprises the centromere regions with the bases of the long and short arms; the second is constituted by the ends of the short arms which are elaborated into a distinctive "frazzle." The two types of chromosomes differ essentially in two simple inversions: one involves a median "blister" indicator (Figure 5 "blister") in the short arm; the second centers on the "shield-triad" sequence in the long arm which is diagrammed in appropriate place and orientation along the chromosomes of Figure 4. Reversal of the banding pattern may be traced beyond the limits of this indicator for a considerable distance in both directions. These two chromosomes will be referred to as "type 1" (left) and "type 2" (right).

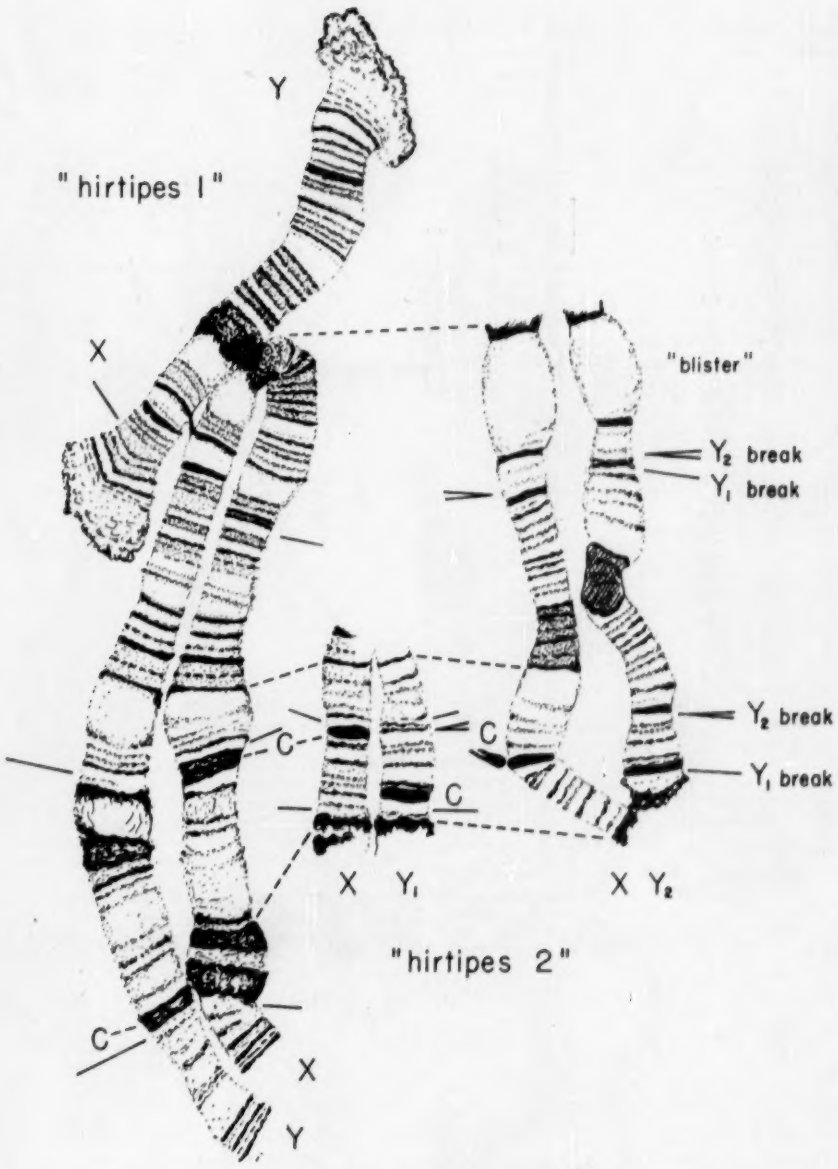
Now, if the classes of individuals which these chromosomes represent are cospecific one would, in a sufficiently large sample, expect to find heterozygotes with typical reversed loops in the centers of the long and short arms of the third chromosomes. The heterozygous inversion in the short arm was promptly found, but it was always accompanied by an additional irregularity in the centromere region and by the type 1 sequence in the long arm. Other heterozygous inversions were



LONG ARMS OF CHROMOSOME III

Figure 4

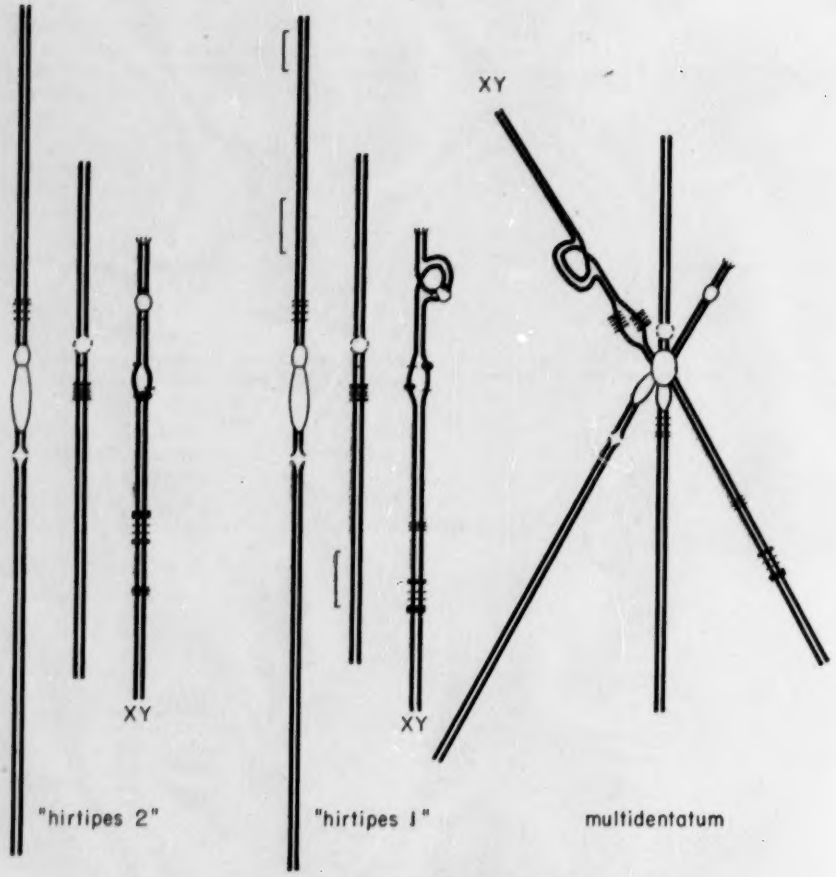
III *l* ends of *Prosimulium "hirtipes* 1" (left) and *P. "hirtipes 2"* (right). Guide lines join homologous bands. The arms differ in a central inversion including the shield (s) —triad (t) indicator region.



SHORT ARMS OF CHROMOSOME III

Figure 5

Short arms (III *s*) of the sex chromosomes of *Prosimulium "hirtipes* 1" (left) and *"2"* (center and right). *C* denotes centromere. Broken lines join homologous bands. Solid lines indicate breaks of rearrangements between X and Y.



IDIOGRAMS OF THREE SPECIES

Figure 6

Salivary gland chromosome idiograms of *P. "hirtipes* 1", *"2"* and *P. multidentatum.*

found in III *s* and these were exclusively associated with the type 2 sequence in III *l*. Although eight different kinds of heterozygous inversions were found in the long arm, the critical inversion which distinguishes the standard chromosomes was not observed in the heterozygous condition. Yet the two homozygous types appeared side by side and at the same time in numerous populations and in hundreds of larvae from the area Toronto, Ottawa, North Bay.

The puzzle cleared up when it was found that all larvae with heterozygous inversions in III *s* were males, and these were of two kinds depending upon the exact rearrangement of the short arm and on the sequence of III *l*. All homozygotes were female and these were either of type 1 or type 2 except for casual inversions superimposed on either type and of no direct concern in this analysis. We thus have two genetically isolated breeding populations, males and females of *"hirtipes* 1", and males and females of *"hirtipes 2"*. Since these forms are sympatric and on the wing at the same time yet do not produce detectable hybrid progeny they are biologically at least distinct species. Their distinctiveness is fully supported by their entirely separate spectra of floating inversions. In calling them siblings we are perhaps unfair to the taxonomist, for the larval, pupal and adult material of the two types has not yet been submitted

to a specialist for study. On the other hand, both forms are common in southern and central Ontario, must have been picked up many times, and been lumped in casual examination.

This is only the first of several instances of sibling species which we have encountered and analyzed in a similar way. Since such instances were not deliberately searched for but thrust themselves on our attention the phenomenon is presumably widespread in black flies.

Sex Determination in "*Hirtipes* 1" and "*Hirtipes* 2"

The differential segments of the X and Y chromosomes of *hirtipes* are shown in more detail in Figure 5. In *"hirtipes* 1" the Y chromosome differs from the X by two inversions in the short arm. The distal one centers on the "blister" indicator, its appoximate breakpoints are indicated on the X chromosome by lines. The proximal one is a short pericentric inversion; its breaks are indicated both on the X and Y chromosome. There is no doubt that the centromere is included; its exact position has been ascertained by specific ectopic pairing in many nuclei. At the other end of this inverted segment a conspicuous "capsule" forms a convenient marker. The two inversions are separated by a substantial normal pairing segment. Crossing-over in this segment should separate the "blister" inversion and the pericentric inversion but neither of these inversions has been found alone. The critical crossover either does not occur or the products of crossing-over are selected against. It is of course possible that X and Y differ by more than the two postulated rearrangements and that crossing-over results in complementary duplications and deficiencies, but there is no evidence for this. An additional type of Y chromosome is known in which the central pairing segment is also inverted in a third step. This inversion overlaps both its "predecessors" by a few bands. It is rare in the populations examined by us.

"Hirtipes 2" has a large number of types of Y chromosomes. Most notable is the fact that both X and Y of type 2 share a terminal homology with type 1 Y rather than X. This homology extends right through the "blister" indicator and may continue all the way down to the centromere region. The simplest Y chromosome is shown in the center of Figure 5 and labelled Y_1. It differs from the X chromosome again in a short pericentric inversion; the short arm break is identical with that of *"hirtipes* 1" but the long arm break is just proximal to the capsule instead of distal. The next common derivative of this Y chromosome (Y_2) is shown on the right hand side of Figure 5. Y_2 differs from Y_1 by a simple inversion which extends up to the "blister" indicator region. Its breaks are marked on X, Y_1 and Y_2 by double lines. This Y_2 inversion overlaps Y_1 by two bands. Further Y chromosomes are derived by additional inversion steps, in the most extreme case discovered so far, resulting in six loops and involving most of the long and the short arms in overlapping steps.

How did all this come about? To be economic of rearrangements the probable history of the development of the sex determining mechanisms in *"hirtipes* 1" and *"hirtipes 2"* must be visualized as follows: The common ancestor had the type 2 sequence in III *s* which still persists in the Y of type 1, in X and Y of type 2, and in two other species of *Prosimulium*. Assuming otherwise, would force the improbable conclusion that the type 2 sequence arose identically and independently twice, once in type 1 to give type 1 Y, and once in the common ancestor to give type 2.

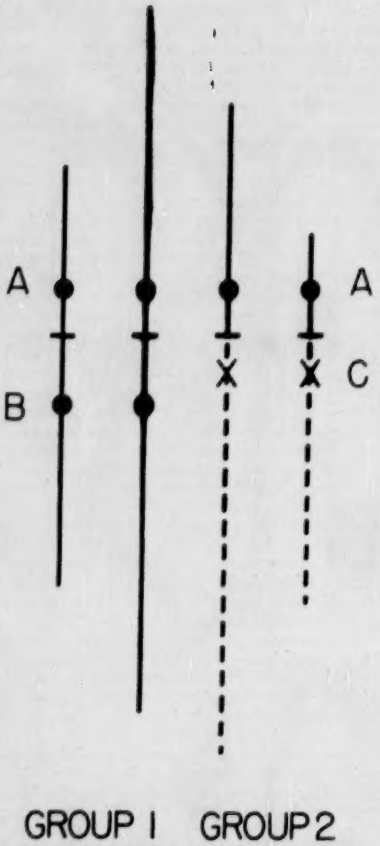
Since it is *a priori* improbable that an inversion as such is the primary sex determining mechanism, we assume that the basic difference between X and Y resides in a minute banding modification which has not so far been detected with certainty. This minute differential segment was secondarily enlarged by inversions, which created genetically isolated blocks on X and Y chromosomes in which modifiers could accumulate to accentuate the primary mechanism. The first steps in both species were short pericentric inversions of almost identical dimensions, suggesting strongly that the primary sex locus is located near the centromere of III. In *"hirtipes 2"* the differential segment was further enlarged by several inversions of the Y chromosome. In *"hirtipes* 1" the next step was an inversion of the "blister" region of the X chromosome which successfully displaced the ancestral arrangement from the female line.

The evolution of the sex determining mechanism in these black flies is identical in all essential respects with that described very recently by Beerman[1] for Chironomids.

Representation of Gross Complement Characteristics in Idiograms

Discovery of an XY mechanism of sex determination in these two species led us to search for it in others. Apart from the intrinsic importance of such mechanisms, the distribution of differential segments would add yet further distinctive idiogram features. Five additional diploid *Prosimulium* species were promptly found to have sex determining mechanisms resembling those of *hirtipes;* and "sex bivalents" entirely similar in principle were discovered in many other forms, including *S. tuberosum* and *S. pictipes.* Yet it is probable that species exist which have preserved the putative ancestral condition of an undetectable differential segment.

We will not describe the sex chromosomes of other species in detail. Figure 6 shows the simplest known XY pair of *P. "hirtipes 1"* and *"2".* It also shows the commonest "sex bivalent" of the chromocentric *P. multidentatum* where the differential segments are lo-



FOUR SPECIES IN TWO GROUPS

Figure 7

Idealized situation where four species fall into two groups according to shared homologies. A, B and C denote specific bands or chromomeres. Horizontal lines designate change in homology between groups. If rearrangements are unique in evolution, the two groups must be monophyletic.

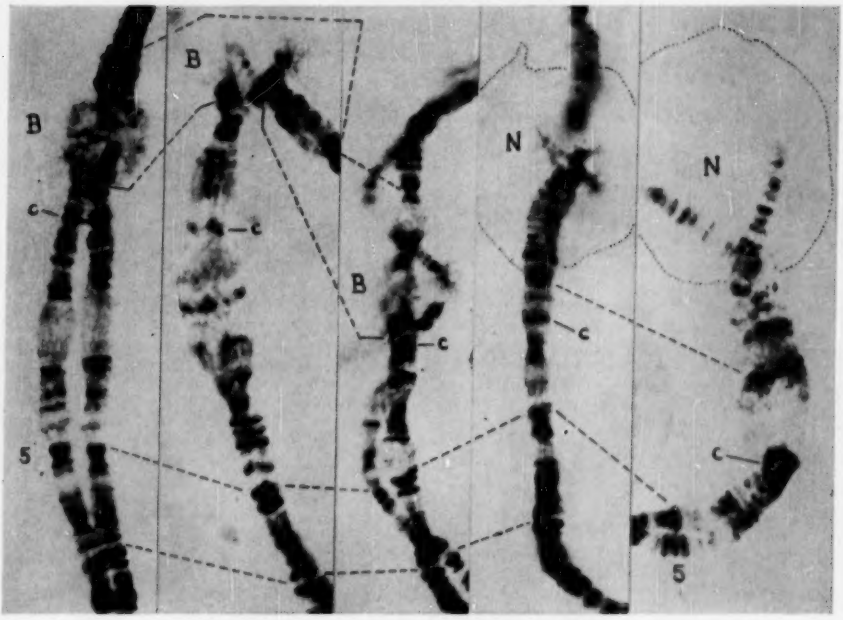
cated in I *s*. Before publishing the over 30 additional idiograms which are on file it may be useful to submit the conventions of their construction to the discipline of criticism by others.

Each idiogram is based on measurements—expressed as percent total complement length (percent TCL)—of a number of nuclei from a number of larvae. The nucleolar gap which stretches differentially is not measured but is represented by an additional one percent length in the diagrams. Chromosome I is always on the left, II in the center, III on the right, in decreasing order of total length. The upper arm is the shorter throughout, and the "short-long" terminology supersedes the "right-left" used previously. Spacing of constituents represents an attempt to show diagramatically degree of pairing. Chromocenters are indicated where present, where absent, centromeres are shown by transverse marks aligned horizontally. Representation of expanded centromere regions is "impressionistic" and not based on actual measurements. Position of the nucleolus is shown by a break (base of I *l*, Figure 6), and Rings of Balbiano by broken circles (base of II *s*, Figure 6). Certain additional landmarks are represented to identify chromosome arms. These include in *hirtipes* the "3 heavy groups" in the base of I *s*, the "group of 5" in the base of II *l*,"frazzle" and "blister" in III *s*, and the "shield-triad" indicator in III *l*. In addition there are shown by brackets the positions of floating inversions, at least regionally useful as indicators of species identity. Finally, as already explained, the pairing configuration of the simplest "sex bivalent" is shown for each species, and the chromosomes labelled XY where changes in the position of landmarks are involved. In the case of the XY pair, proper percent TCL is obtained by measuring straight from the centromere to the end, and not by following out loops.

For mapping purposes and numbering of bands, the ternary *Drosophila* system as adapted[6] to black flies is followed. We now divide the complement into 100 primary sections, 1, 2, . . . 100, beginning with I *s*, I *l*, II *s* . . . through to III *l*. On this system, once arm length is known in percent TCL, partial maps of preliminary studies may be accommodated into the definitive complete map without change of symbols. The nomenclatorial system is open. Even where interspecific homologies are quite obvious we have adhered to a separate choice of section limits for each species. For purposes of comparison it is always possible to number sections *ad hoc* in terms of a related species. This procedure reveals simultaneously serial homologies between species and the distribution of recognized interspecific rearrangements.

Grouping of Species

On the basis of idiogram features all species of *Gymnopais, Prosimulium, Cnephia, Eusimulium* and most species of *Simulium* can readily be separated, and dichotomous keys might be drawn up. Such artificial keys are, however, of limited interest for they tell us little about phylogenetic relations. The cytological tool which is of help here is the "principle of the evolutionary uniqueness of rearrangements". If chromosome breaks are rare and random and the two breaks of a rearrangement may occur equally well at any one of several thousand sites, the probability of



ASSOCIATION OF THE "GROUP OF 5"

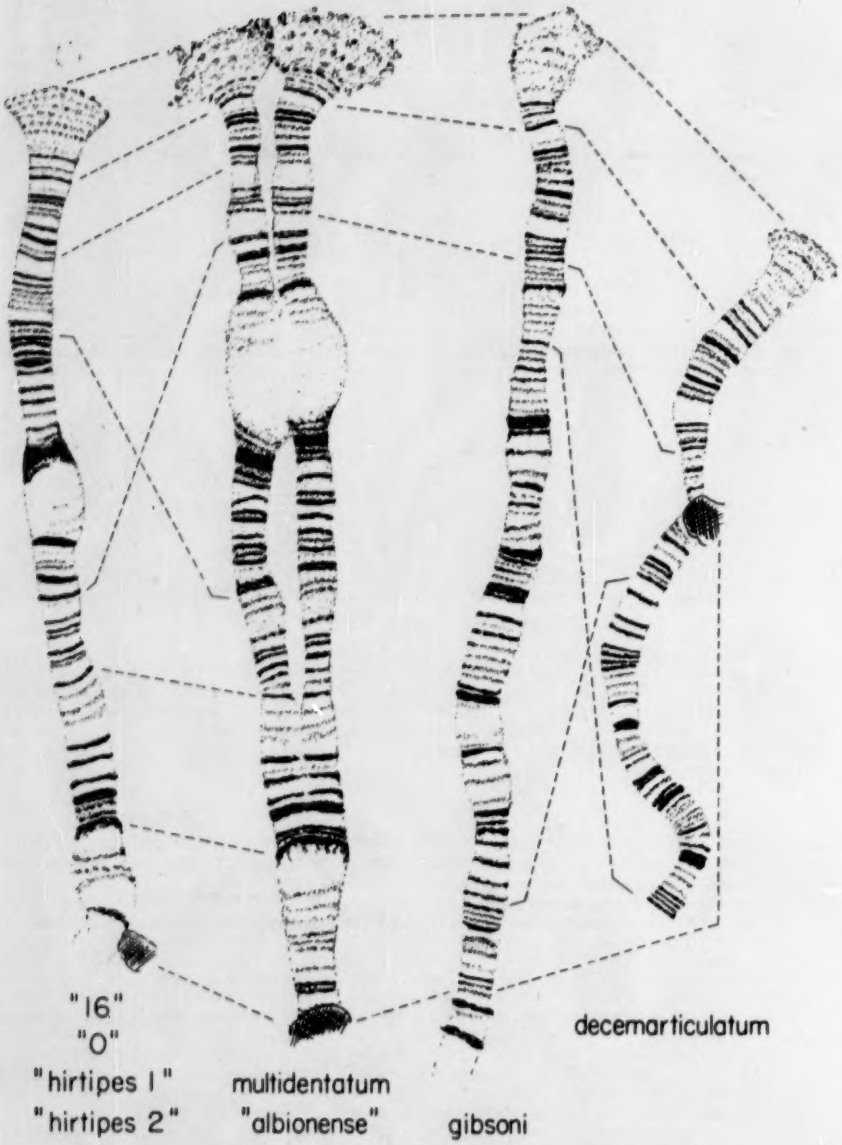
Figure 8

The association of the "group of 5" with the Ring of Balbiani is shown in three species of *Prosimulium* (from the left: *"hirtipes* 2," *gibsoni, "0"*); and the association of the "group of 5" with the nucleolus in two other species (from the right: *multidentatum, "albionense"*). Broken guide lines connect homologous landmarks. 5 denotes "group of 5," B the Ring of Balbiani, N the nucleolus and C the centromere.

the repeated independent occurrence of the identical rearrangement, is vanishingly small. It follows that species which share a specific banding sequence are more closely related to one another than to species which share a different sequence, provided it overlaps the first. This reasoning has been set forth vigorously and in greater detail by Dobzhansky and others. Certain qualifications which must be made, particularly in regard to clustering of spontaneous breaks,[6] do not invalidate the method; they force us, however, to ascertain the uniqueness of rearrangements by rather more exact determination of breaks.

The principle has already been tacitly invoked when reconstructing the evolution of the sex determining mechanism in *P. hirtipes*. It may be applied in various ways but the most general, directly useful, situation is that diagrammed in figure 7, where several related species fall into two groups depending upon whether they share sequence AB or AC. If the change of homology between groups is closely bracketed one can stipulate polyphyletic constitution of groups only by invoking improbable coincidences of breaks. Of these the repeated occurrence of the identical two-break rearrangement is the least extreme.

The following eaxmples illustrate the application of the method. Comparative studies show that with one exception the band content of chromosome arms is preserved through six arms and eight species of *Prosimulium*. The exception concerns a pericentric inversion in III *s* of *P. decemarticulatum*, to be described later. However, while the essential homology of arms is preserved, species fall into two groups on the basis of the combination of arms into chromosomes. In species of group 1 (all except *multidentatum* and *"albionense"*) the arms characterized respectively by the "3 heavy groups" and the nucleolus are associated in chromosome I, while the arms identified by the Ring of Balbiani and the "group of 5" are joined in chromosome II (Figure 6). in *multidentatum* and *"albio-*



SHORT ARMS OF CHROMOSOME III IN EIGHT SPECIES

Figure 9

The figure shows the short arms of the third chromosomes of eight species of *Prosimulium*. The species fall into four groups on the basis of shared rearrangements. Homologous bands are connected by broken guide lines.

nense" (group 2) the "group of 5" is associated with the nucleolus in chromosome I, and the Balbiani Ring with the "3 heavy groups" in chromosome II (Figure 6). Thus groups 1 and 2 differ by "whole arm transfers" with interchange breaks close to centromeres I and II. Figure 8 shows the association of the "group of 5" with the Ring of Balbiani in *"hirtipes 2," gibsoni,* and the triploid *"O"* (group 1); and the association of the "group of 5" with the nucleolus in *"albionense"* and *multidentatum* (group 2). Centromeres are also indicated as identified by ectopic pairing (*hirtipes*), or breaking of chromocenters (*multidentatum*). Certain additional landmarks are joined by guide lines to substantiate homologies of arms. Centromere regions I and II are strictly homologous within groups (with certain exceptions in group 1 that are not relevant to the present argument) and differ of course between groups. This intra-group identity of centromeres proves the uniqueness of the interchange by which the groups differ, and establishes, on accepted principles, the monophyletic origin of the groups themselves.

Repeated application of the principle allows the subdivision of major groups into smaller segregates. Thus one may further group the *Prosimulium* species by comparing the III *s* ends as shown in Figure 9. On this basis one obtains four groups. The first comprises *"16"* (shown in the drawing), *"hirtipes* 1" (if Y distal and X proximal are combined), *"hirtipes 2,"* and with minor modifications *"O."* These four species have essentially the same banding sequence through the arm. A second group comprises *multidentatum* (shown in drawing) and *"albionense."* They are homologous band for band. They share terminal and basal homologies with group 1 (guide lines) and differ essentially by a central inversion involving the "blister." This common inversion associates *multidentatum* and *"albionense"* with each other and separates them from all others, thus corroborating the conclusions previously founded on a common interchange. Groups 3 and 4 represented by *gibsoni* and *decemarticulatum* are best considered together. They share a substantial terminal homology, especially with group 2, but the proximal sequence is scrambled to such an extent that no major segment can be homologized with species of other groups. On the other hand, *gibsoni* and *decemarticulatum* differ from each other only by a simple pericentric inversion; they share band for band the entire rescrambled basal part of the arm, and are therefore more closely related to each other than to other known *Prosimulium.*

This line of reasoning does not indicate which arrangement is the ancestral one. One may, with almost equal justification, pick any one of the four groups as most closely resembling the ancient type. Direction signs will have to come from somewhere else, and the evolution of sex determining mechanisms of increasing complexity may provide one such source.

The method may be applied at the generic level just as surely as at the specific. Thus in the *Eusimulium-Simulium* complex, the generic separation of which is contraversial, two kinds of II *l* ends are common; one of these has been found in 12 species—all *Eusimulium,* the other in 11 species—all *Simulium.* While this does not settle the generic status of either segregate, it does show that the 12 *Eusimulium* studied belong together in one group and the 11 *Simulium* in another.

It has proved possible to homologize one chromosome end—I *s*—through all five genera, and there is distinct hope that banding comparisons may extend fruitfully through the entire family once sufficient forms are studied. Only a very minor fraction of the potential represented by the entire chromosome complement has so far been exploited. Since the procedure is rational, repeated application of the principle should reveal any fallacies in methods and assumptions by giving contradictory and inconsistent results. If this does not occur, the first convincing notions will emerge of phylogenetic affinities within the family of black flies.

Summary

Fixed banding differences, diverse spectra of floating inversions, and differential sex determining mechanisms are assessed for their value in revealing sibling species.

The cytology of sex determination is described for *Prosimulium hirtipes* and sibling. It is postulated that a minute differential segment became secondarily enlarged by successive inversions.

Conventions are proposed for the construction of salivary gland chromosome idiograms and for the nomenclature of chromosomes, chromosome arms, sections and bands.

The use of rearrangements as tracers of phylogenetic affinities is illustrated by examples from the genus *Prosimulium.*

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MULTIPLE NEUROFIBROMATOSIS

THE recent, ever-growing application of human genetics in the fields of clinical medicine and epidemiology is gradually bringing about a necessary collaboration of these disciplines on a close and equal basis. The authors of this monograph,* a competent team of workers from the University of Michigan Heredity Clinic, express the hope that clinical and genetic approaches may be combined in the study of a given disease with profit to both. Rightly so, and their notable contribution is most welcome. In the case of multiple neurofibromatosis, however, it should be borne in mind that the clinical and pathological aspects have been well worked out and are described in all modern textbooks of clinical neurology and pathology. One of the main tasks of the workers here would be that of integration of the old observations with the new.

The material for this study was collected mostly from hospitals and institutions in the state of Michigan. It consists of 137 propositi and 86 affected relatives distributed among 107 families. This is somewhat unusual, for in human genetics it is customary to recognize one propositus for each family. According to the authors no family histories were collected ror 20 institutionalized patients but the reviewer was not able to trace the fate of the remaining 10 propositi.

The book is divided into 1) Clinical aspects, 2) The pathology of neurofibromatosis, 3) Description of the clinical material and 4) Genetics of neurofibromatosis.

In describing and discussing the clinical aspects of the disease as seen in their material, the authors mostly agree with the descriptions of previous workers. In addition, a very conscious effort is made at integration and evaluation of some of the material from the clinical and genetic points of view, as in the cases of intra-family similarities in tumor site, and the café-au-lait spots. These latter pigmented areas have always been used as a diagnostic aid in multiple neurofibromatosis but to the knowledge of the reviewer this is the first attempt at statistical treatmcnt of the frequency and occurrence of the spots in affected persons and the general population with a view to using them as a more or less accurate diagnostic criterion. The authors consider only those spots whose broadest diameter equals or exceeds 1.5 centimeters in diameter. This standard is indeed arbitrary, for even smaller café-au-lait spots are not easily confused with other pigmented areas of different nature. The authors find that 94 percent of their particular sample of patients had at least one spot and 78 percent had six or more spots. They conclude that any person with more than six spots must be presumed to have multiple neurofibromatosis even in the absence of positive family history, and approximately 80 percent of affected persons can be diagnosed solely on the basis of the café-au-lait spots present.

The discussion of the pathology of neurofibromatosis is confined to five pages with a promise that the pathological studies will be presented elsewhere. This brief summary would hardly justify the inclusion of the word "Pathological" in the title of the book. In general, the authors agree with descriptions and explanations given by previous workers. They stress the diagnostic importance of mast cells which they claim to be a constant finding in their series, especially in the Antoni Type B tissue. In contrast, they state that mast cells are virtually absent from their series of lipomas and leiomyomas and other tumors "easily confused with neurofibromas." Most pathologists would take issue with this statement. Neurofibromas are not obscure tumors, as the authors refer to them, and are not easily confused—pathologically at least—with lipomas and leiomyomas. Mast cells are seen frequently as a normal component of areolar connective tissue which serves as stroma for the rather loose, acellular, non-anaplastic type of neurofibroma. Such tissue may resemble the Antoni Type B tissue although again, this terminology is used in connection with neurilemmoma rather than with neurofibroma. The photomicrograph of perivascular mast cells published in the text tends to justify this impression. The diagnostic significance of mast cells cannot be fully reviewed and evaluated unless a detailed description of the pathological study is available.

The description of the clinical material is quite extensive and takes up half the pages of the book.

The genetics of multiple neurofibromatosis receives short but comprehensive treatment. The cases are divided into familial and sporadic and although the terms are well defined, one cannot help but feel that these terms ought to be eradicated from the genetic literature. In the medical literature they have been

(*Continued on page* 128)

**A Clinical, Pathological and Genetic Study of Multiple Neurofibromatosis.* F. W. Crowe, W. J. Schull and J. V. Neel. Charles C. Thomas, Springfield, Illinois. 181 pp. 1956.

STEEL, A NEW DOMINANT GENE IN THE HOUSE MOUSE

With Effects on Coat Pigment and Blood

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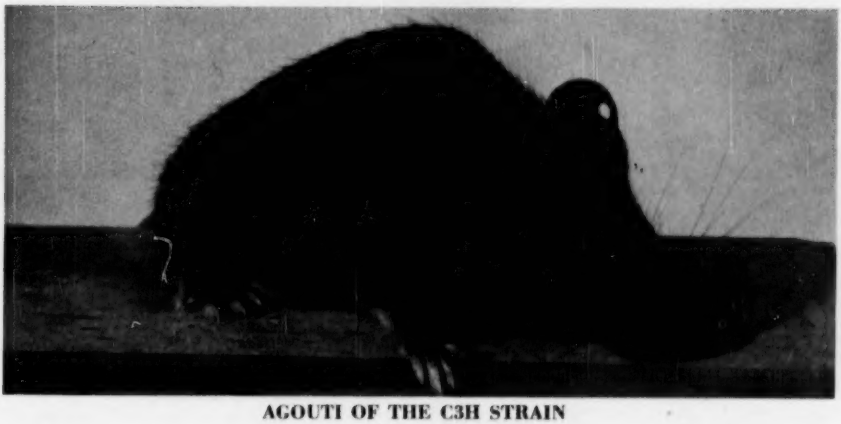

AGOUTI OF THE C3H STRAIN

Figure 10

Seventeen agouti young of this type appeared in a litter with 22 of the new mutant, Steel.

A SINGLE female of a somewhat diluted fur color and with a small white spot on forehead, belly, and tip of the snout occurred in the eighth generation of brother-sister mating in this laboratory of the inbred C3H strain, obtained from W. E. Heston in 1948. When mated to a wild-type littermate, this female produced 22 (12 ♂, 10 ♀) young similar to herself in appearance and seventeen (9 ♂, 8 ♀) agouti young. The new type was named Steel (*Sl*) and has been maintained in a brother-sister inbred subline of the C3H strain, which may be given the strain symbol C3H Sl.

Genetics

Penetrance and Expressivity

Sl is a dominant, as proved by crosses of Steel animals to unrelated C3H (yielding 121 Steel to 120 agouti), and by outcrosses of Steel to other strains (see below). The good 1:1 ratio from $+/+ \times Sl/+$ and $Sl/+ \times +/+$ crosses within the C3HSl strain (Table I) indicates that $Sl/+$ is normally viable and that penetrance on the C3H background is complete. This latter conclusion also finds support from the tests of 22 agouti offspring of Steel parents (footnotes to Table I): not one of these agoutis was found to transmit Steel.

On the C3H background the Steel phenotype is recognizable by the following externally visible features (see also Figure 11 and compare with Figure 10): (a) slight over-all dilution of the fur color, more extreme on the belly than on the back; (b) light ears, feet, vibrissae, and tail; (c) a white snout tip; (d) almost invariably a small white spot in

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THE STEEL MUTANT
Figure 11

On the C3H background the Steel (*Sl*/+) mutant can be recognized by a slight over-all dilution of the fur color, light ears, feet, vibrissae and tail, a white snout tip and usually a small white spot in the middle of the forehead and on the belly.

the middle of the forehead, and, almost as frequently, one on the belly (however, non-Steel segregants may occasionally, though very rarely, also have a small belly spot); and (e) very occasionally a white blaze between the eyes or a few white hairs on the back. Steels of both sexes are normally fertile and the females make good mothers (Table II).

Steel males were outcrossed to females of six other stocks (C57 Ln, C57 Sp, Rus, *ru/ru;si/si;s/s*, (101 × C3H)F_1, *a/a; b/b;pc^{ch}/pc^{ch};dse/dse;s/s*) and 120 young obtained. Of these, 69 were agouti and 51 had somewhat diluted fur color (difference from 1:1 ratio not significant). The fur color dilution was occasionally very slight and was not always accompanied by the pattern of small white spots present in Steels on the C3H background, the head spot being absent most often, the belly spot least often. In view of the occasionally greatly decreased expression of Steel, it is possible that the slight (though not significant) excess of agoutis in the outcrosses may represent incomplete penetrance on some backgrounds. The 69 agouti offspring of the outcrosses were not, however, systematically tested to detect such a situation, tests being confined to those few which had small belly spots. (Belly spots in the absence of color dilution were found to be attributable to the background—*Ss*, or C57BL—rather than the Steel gene.)

Steel by steel matings in the C3HSl strain (Table I) produced no new type and only 63 percent of the mutant type instead of the 75 percent which should have been found on a background where there is complete penetrance (as there is in C3HSl) if the homozygote were viable. This type of mating, moreover, yielded a littersize only 71 percent of normal at birth (Table III). It was, therefore, concluded that *Sl Sl* animals

TABLE I. Offspring of various types of mating in the C3H Sl strain, classified at weaning age

Type of mating ♀	♂	Steel ♂♂	Steel ♀♀	Agouti ♂♂	Agouti ♀♀
+/+	× *Sl*/+	218	200	202*	205*
Sl/+	× +/+	34	49	38	40
Sl/+	× *Sl*/+	103	110	59†	65†

*Of these 2 ♂♂ 9 ♀♀ tested and found not to transmit Steel.
†Of these 4 ♂♂ 7 ♀♀ tested and found not to transmit Steel.

TABLE II. Comparison between Steel and agouti mothers* with regard to size of litters raised to weaning age

Type of mating ♀	♂	No. of matings	1st litter	2nd & 3rd litters
Sl/+	× +/+	9	5.33	5.85
+/+	× *Sl*/+	9	5.22	5.87

*Mated at comparable times.

DOUBLE HETEROZYGOTE

Figure 12

This type ($Sl/+;W^v/+$) is very heavily mottled dorsally and completely white ventrally. The remaining hairs on the back are much lighter than in either $Sl/+$ or $W^v/+$.

probably die *in utero*, and dissections were undertaken to verify this point. (Embryology will be discussed below).

Allelism tests with W^v

Because of their general similarity in effect, *Sl* and W^v were tested for allelism. The cross $W^v/+ \times Sl/+$ (made in either direction) produced, in addition to agouti, $W^v/+$, and $Sl/+$, a new type (Figure 12) which was very heavily mottled dorsally and completely white ventrally. The remaining pigmented hairs on the back were considerably lighter than in either $W^v/+$ or $Sl/+$. This new type was assumed to be either the compound or the double heterozygote. Among 225 F_1 offspring, there were 57 light mottled (Table IV), which is as expected if this type is fully viable. It should be noted that W^v and *Sl* apparently interact, rather than being merely additive, at least with respect to pigment dilution. Light mottled animals of both sexes are fertile.

Under the assumption of allelism, F_2 (light mottled by light mottled) should yield only black-eyed whites and light-mottled animals. However, among 61 F_2 offspring raised, there were 15 which were single heterozygotes and two agouti. This proves *Sl* and W^v to be separate loci.

Light-mottled animals were tested for possible linkage between *Sl* and W^v. In the tabulation (Table IV), $Sl/+;+/+$ and $+/+;W^v/+$ progeny were lumped since they usually were phenotypically

TABLE III. Average litter size at birth

Type of mating ♀ ♂	Number of Mothers	Litters	Average number of young per litter
$+/+ \times Sl/+$	28	92	6.62 ± 0.16
$Sl/+ \times Sl/+$	20	81	4.72 ± 0.20

TABLE IV. Results of crosses between Steel (*Sl*) and Dominant Spotting (W^v)

Mating	Offspring: Light mottled	$+/+;W^v/+$ and $Sl/+;+/+$‡	Agouti	Black-eyed white
$+/+;W^v/+ \times Sl/+;+/+$*	57	111	57	—
Lt. mottled × Lt. mottled	27	15	2	17
Lt. mottled × Agouti, repulsion*	119	231	138	—
Lt. mottled × Agouti, coupling*	53	109	60	—

*No significant differences were noted between reciprocal matings.
†Light mottled F_1 animals were crossed to agouti segregants of the Steel strain.
‡These two types are often phenotypically indistinguishable and were lumped in classification.

indistinguishable from each other. Neither in the repulsion nor in the coupling cross do the ratios depart significently from the 1:2:1 that is expected on the basis of independent assortment ($P>0.2$ and $P>0.7$, respectively). Summing results of the two crosses, those segregants that—were there linkage—would constitute the "non-crossover" group (single heterozygotes from the repulsion cross; agoutis and light-mottleds from the coupling cross) number 344, and the "crossover" segregants number 365. It may therefore be concluded that *Sl* assorts independently of W^v.

The data in Table IV shed some light on the question of whether the type W^v/W^v; *Sl*/+ is viable. No new phenotype was found among the offspring of the light mottled by light mottled cross and, if W^v/W^v; *Sl*/+ animals are viable, they must be among the black-eyed white segregants which cannot be tested because of sterility. On the assumption that W^v/W^v; *Sl*/+ is viable, the expected number of black-eyed whites among the 61 F_2 offspring is 15.25; if it is lethal, the expected number is 5.08. The actual number of 17 black-eyed whites would seem to indicate that W^v/W^v; *Sl*/+ was viable.

Embryology

To determine the time and, if possible, cause of death of the animals missing at birth, i.e., the presumed *Sl/Sl* type, uterine dissections were carried out in 59 Steel females mated to Steel males. A control group consisted of 21 Steel females mated to agouti males. Results are shown in Table V.

It should first be noted that there is no difference between the two types of mating with regard to preimplantation death: the percentage of corpora lutea not represented by implants was 12.0 percent in *Sl*/+ × *Sl*/+ and 12.1 percent in *Sl*/+ × +/+ matings. It may, therefore, be assumed that preimplantation deaths are randomly distributed among the segregating genotypes, and calculations from here on will be based on total implants rather than corpora lutea.

Abnormal embryos were of two types: 1) a relatively small number, scattered through various age groups, showing malformations of the central nervous system (see footnotes to Table V for details); 2) a large group, occurring within relatively circumscribed age limits, characterized by grossly recognizable anemia. The incidence of central nervous system abnormalities was higher in the *Sl*/+ × *Sl*/+ matings but not significantly so. For comparable age groups, i.e., days 14½-17½ inclusive, there were seven among 330 morphologically observable embryos of *Sl*/+ × *Sl*/+ matings and none among 166 embryos of *Sl*/+ × +/+ matings ($t = 1.9$). Attention will, therefore, be centered on the anemic embryos as probably including the presumed *Sl/Sl* genotype.

In *Sl*/+ × *Sl*/+ matings, no anemics were noted in gross observations of 42 living em-

TABLE V. Observations on embryos of *Sl*/+ × *Sl*/+ and *Sl*/+ × +/+ matings

Type of mating	Day post-conception observed	Total Number of: Litters	Corpora lutea	Implants	Resorbing moles	Recently dead	Living and anemic	Living, non-anemic	Percentage of Implants: In advanced resorbtion	Recently dead	Total dead	Alive and anemic[a]
Sl/+ × *Sl*/+	10½	3	31	29	3	3	0	22 + 1[b]	10.3	10.3	20.7	0
	11½	2	18	17	7	0	0	9 + 1[c]	41.2	0	41.2	0
	12½	2	15	11	2	0	0	8 + 1[d]	18.2	0	18.2	0
	13½	6	53	49	11	2	3 + 3[f]	30	22.4	4.1	26.5	12.2
	14½	11	117	102	23	4	18 + 7[f]	50	22.5	3.9	26.5	24.5
	15½	13	127	119	18	10 + 2[e]	11 + 4[f] + 2[f]	72	15.1	10.1	25.2	14.3
	16½	10	103	88	18	12 + 2[g]	1	55	20.5	15.9	36.4	1.1
	17½	12	121	100	20	14 + 1[h]	1 + 3[f]	61	20.0	15.0	35.0	4.0
Sl/+ × +/+	14½	4	37	33	4	0	0	29	12.1	0	12.1	0
	15½	5	54	45	7	2	1[f]	35	15.6	4.4	20.0	2.2
	16½	9	96	86	10	3	1[f]	72	11.6	3.5	15.1	1.2
	17½	3	28	25	2	1	2[f]	20	8.0	4.0	12.0	8.0

[a]Includes doubtfully anemic embryos.
[b]Collapsed brain with scalloped edges.
[c]Pseudencephaly.
[d]Knob on top of head, brain region narrow and convoluted.
[e]One with spina bifida; one with pseudencephaly.
[f]One with spina bifida; one with spina bifida and pseudencephal.
[g]One with spina bifida; one with collapsed brain region.
[h]Clear bleb slightly to midline in cervical region.

bryos aged 10½, 11½, or 12½ days (Table V). From days 13½ to 15½ the frequency of anemic embryos is high, with a peak on day 14½ where it reaches the expected 25 percent, but by day 16½ it has dropped to the level of the controls. (Anemic embryos in control, *Sl*/+ × +/+ matings will be discussed below.) Anemia is recognized not only by the over-all paleness of the embryo but, more reliably, by observation of the blood flowing through the umbilical vessels. In normal embryos, these vessels are uniformly red; but in affected animals, it is possible to see individual clumps of red cells (or perhaps even individual cells?) moving in the bloodstream. The anemia appears to increase in severity with age of the affected embryo. Measurements of crown-rump lengths at 14½ and 15½ days showed that the anemic animals did not have retarded growth (Table VI).

In the four age groups of the *Sl*/+ × +/+ control matings, recently dead embryos average 3.2 percent of the implants. It is clear that, in *Sl*/+ × *Sl*/+ matings, the "recently dead" group is markedly increased beginning at least with day 15½. The sum of anemic plus recently dead embryos (subtracting, in each case, the appropriate control figure) is as follows: about 13 percent on day 13½, which indicates that not all of the anemias have become grossly recognizable by that time; about 25 percent on day 14½, by which time presumably all *Sl*/*Sl* embryos have expressed their anemia but few, if any, have died as yet; about 19 percent on day 15½, by which time a certain amount of death of anemics has already occurred; and 13 percent and 12 percent on days 16½ and 17½, respectively, when all, or virtually all, *Sl*/*Sl* anemics are presumably dead. The results for days 16½ and 17½ are far short of the expected 25 percent. This may be remedied by using total deaths, rather than recent deaths alone, in the calculations, i.e., by assuming that there is very rapid resorption of dead anemic embryos. This device, however, leaves very much to be desired. In the first place, it is difficult, on *a priori* grounds, to conceive of such rapid resorption so late in pregnancy; so rapid, in fact, that the change in classification from "recently dead" to "resorbing mole" would have to be achieved in one day. Secondly, the use of total rather than recent deaths raises new difficulties in the results of somewhat earlier stages. The mode of death and resorbtion of *Sl*/*Sl* embryos will therefore have to be further clarified by additional experiments.

TABLE VI. Crown-Rump lengths of embryos from *Sl*/+ × *Sl*/+ matings*

Days post-conception observed	Non-anemic embryos		Anemic embryos	
	No. of embryos	Av. length (mm.)	No. of embryos	Ave. length (mm.)
14½	29	11.3 ± .21	17	11.0 ± .42
15½	61	13.9 ± .25	13	13.7 ± .54

*All embryos were alive at the time of measurement.

The "probably anemic" animals in the *Sl*/+ × +/+ control matings (marked "?" in Table V) were assumed to be *Sl*/+. To test this assumption at least partially, litters from *Sl*/+ × +/+ as well as *Sl*/+ × *Sl*/+ matings were observed within a few hours after birth. It was possible to discover several young which were definitely paler than their littermates. These were marked at the time and when tested later all proved to be *Sl*/+. Determinations of this type were not done on a large enough scale to establish whether grossly anemic *Sl*/+ young are more frequent at birth than *in utero*, as seems indicated by the increase in frequency of *Sl*/+ anemics with fetal age. In other litters (which had not been observed for the presence of pale newborns), a few exploratory blood counts were made of *Sl*/+ and +/+ littermates after the age at which classification by coat becomes possible (Table VII). It may be seen that in four comparisons at 7-13 days of age the red counts are 20-30 percent lower in Steels than in agouti littermates. (White counts are inconsistent in the present data.) It may, therefore, be concluded that Steel heterozygotes are slightly anemic, at least until 13 days of age, and that, occasionally, this anemia may be severe enough to be detected grossly as early as 15½ days postconception. It would be of interest to determine: 1) whether the variation among *Sl*/+ animals is only in the time of onset of their anemia or also in the degree of anemia finally reached; and 2) whether the anemia is of a transitory nature. At any rate, even those *Sl*/+ animals which are anemic enough to be grossly diagnosed at birth are apparently viable.

Discussion

Even a casual comparison reveals similarities between *Sl* and the mutants at the *w*-locus[1,3]: in both cases the effects are on coat pigment as well as on the blood picture. In their effects on the coat, *Sl* and W^v resemble each other even further in that each of them, in heterozygous condition, produces spotting as well as general slight dilution of color. The fact that, in the double heterozygote, *Sl*/+; W^v/+, the two genes appear to interact with respect to both spotting and dilution would seem to indicate that they affect similar processes.

Both *Sl* and W^v are semi-dominant with respect to the anemia. While *Sl*/+ and W^v/+ are apparently equally viable, *Sl*/*Sl* animals are much more severely affected than W^v/W^v

TABLE VII. Blood counts in *Sl*/+ young and +/+ littermates

Age in days	+/+		*Sl*/+	
	White	Red	White	Red
7	5,140	4,485,000	5,540	3,550,000
7	5,720	4,455,000	5,680	3,125,000
8	4,200	6,480,000	4,980	5,250,000
13	7,640	6,260,000	4,240	4,465,000

and even more severely than *W/W*, whose death is entirely postnatal. The comparison of the anemias cannot be further pursued at this time, since no detailed haematological studies of Steel homozygotes or heterozygotes have been made to date.

In *Sl/Sl* embryos, anemia is first grossly recognized at 13½ days, and has presumably become expressed in every *Sl/Sl* by 14½ days. The liver starts acting as a haemopoietic organ on the 13th day of embryonic life.[1] If it can be assumed that gross manifestation of anemia gives at least a fair indication of the real time of onset of the haematological disorder, then it may be tentatively concluded that the disturbance in *Sl/Sl* embryos is in some way associated with liver haemopoiesis and it may be predicted that abnormalities of the intermediate rather than the primitive blood cells will be found in anemic *Sl/Sl* embryos. Whatever the nature of the disturbance, it is evidently of a more severe nature than in flexed tail, *f*.[1,2] In *f/f* animals there is a recovery from anemia when the intermediate cells are replaced by definitive red cells, but *Sl/Sl* embryos die before the bone marrow even starts its haemopoietic function. It will be of interest to study *Sl/+* heterozygotes with a view to determining whether their anemia is transitory.

Summary

A dominant mutation, Steel (*Sl*) arose in the C3H strain. Heterozygotes are characterized by slight dilution of fur color, light ears, feet, tail and vibrassae, and a number of small white spots (tip of snout, forehead, belly) whose occurrence depends somewhat on genetic background. Homozygotes die prenatally, probably on days 15-16 postconception. They are characterized by a severe anemia which is first grossly recognizable on day 13½. Heterozygotes have a reduced red cell count but are viable.

Sl is not an allele of W^v, but the two genes interact in their effects on the coat. *Sl* and W^v assort independently.

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MULTIPLE NEUROFIBROMATOSIS

(*Continued from page* 122)

used to mean different things at different times and abused to the point where they can only cause confusion to many an eager geneticist or physician. Surely, some well-defined, functional terms can be agreed upon.

The authors estimate the frequency of neurofibromatosis from the Michigan data at one in 2,500 to 3,300 births. The fertility of persons affected with the disease, calculated by the Krooth method, is found to be surprisingly low, with that of the males reduced to less than 50 percent.

Multiple neurofibromatosis has long been known to behave as a dominant trait. From analysis of their familial cases the authors find "no evidence that the dominant gene responsible for neurofibromatosis does not enjoy a high level of penetrance." The sporadic cases are assumed to represent new mutations and for some cases the authors present evidence that this is so.

The authors also attempt to calculate a mutation rate for this gene from their material. This, they estimate at 1×10^{-4}, which admittedly is the highest rate for a dominant gene known. The pitfalls and biases involved in the calculation of a mutation rate are numerous and difficult to control and the authors are the first to admit it. In the present case, the mutation rate was estimated under the assumption that the gene had complete penetrance. Yet, previous studies have shown it to skip generations occasionally or favor one sex over the other. Not the least of the biases which may raise the estimate of a mutation rate is the failure to investigate extensively the family histories of the propositi branching into second and third degree relationships.

In general, the material is well presented and the conclusions well documented. As an attempt at integrating the disciplines involved, the book is not altogether successful, but it is a worthy effort and we hope that other monographs of this design will soon follow.

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REDUCED GENE TRANSMISSION IN RADIATION-INDUCED MUTANT BARLEY

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THE present paper is a continuation of previous reports on barley mutants induced by atomic-bomb and X-radiation. These reports[4,5,6] described several lines of barley which produced an unusually high frequency of chlorophyll-deficient mutants and discussed the genetic mechanisms for such abnormal segregation ratios. This report presents results of genetic studies of barley lines which produced a distinctly low frequency of chlorophyll-deficient mutants following self-fertilization. The mechanism responsible for the low frequency of gene transmission is also discussed.

Material and Methods

Seventeen chlorophyll-deficient mutant lines of barley (*Hordeum vulgare* var. Trebi, $2n = 14$) were chosen for cytogenetical analysis. Nine of the mutants were induced by atomic-bomb radiation (Bikini, 1946) and eight were induced by X-radiation. Details concerning methods of irradiation and of handling the material in the X_1 generation have been described by Smith.[7]

The X_2 to X_7 generations were grown at Pullman, Washington. Plants of each mutant line were grown in the field. Progeny derived from self-fertilization of these plants were grown in the greenhouse. The numbers of normal and the chlorophyll-deficient seedlings were recorded within each plant progeny when the first leaves of the seedlings were fully developed (about 12 cm. high).

Heterozygous plants of the mutant lines were reciprocally crossed with Compana, a normal two-rowed variety, to test the transmission of the mutant genes through male and female gametes. The selfed progeny of the testcross plants were grown in the greenhouse.

Results

Cytogenetical Studies

The 17 lines involved the following mutant seedling characters: white, dwarf white, yellow, cream, yellow-green, and pale green. All of the 17 mutant lines produced less than 10 percent mutant seedlings following self-fertilization. Among the surviving green plants, the percentage of heterozygotes for all lines but one (50-996) was significantly lower than 67 percent. A summary of the genetic characteristics of these lines is presented in Table I.

No visible chromosomal aberrations were observed at meiosis in the pollen mother cells of any of the mutant lines. Pollen grains and fertility were apparently normal. The percentage of seed germination for all mutant lines was very high. The seed of all mutant lines germinated higher than 90 percent (Table I).

Transmission of mutant gene through male and female gametes

Reciprocal crosses with Compana to test the transmission of the mutant gene through male and female gametes were successfully made for two lines of mutants (51-915 and 51-917). The results of these crosses are presented in Table II. In both lines the mutant gene was transmitted by about 50 percent of female gametes, but only about 10 percent of the male gametes.

The percentage of mutant seedlings which arose following self-fertilization of the testcross plants was also determined. The average number of mutants from plant 51-915 × Compana was 2.4 percent; from plant 51-917 × Compana, 6.4 percent. Thus, the testcross plants

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TABLE I. Genetic characteristics of mutant lines of barley producing a low frequency of seedling mutants

Progeny number	Source of mutation	Mutant seedling character	Seed germination	Mutant Seedlings: Total Hetero-zygous plants studied	Mutant Seedlings: Total seedlings counted	Mutant seedlings		Total Plants studied	Surviving green plants: Hetero-zygous plants		Surviving green plants: Homo-zygous plants		χ^2
			%	No.	No.	No.	%	No.	No.	%	No.	%	
51-911	X-radiation	Yellow-green	98	57	4,488	170	3.8	121	57	47	64	53	20.90
51-912	X-radiation	White	96	107	13,648	792	5.8	188	107	57	81	43	8.01
51-913	X-radiation	Dwarf white	98	64	8,526	457	5.4	175	64	37	111	63	71.44
51-914	X-radiation	Yellow	98	72	9,585	672	7.2	140	72	51	68	49	14.73
51-915	X-radiation	Dwarf white	99	74	10,071	498	4.9	150	74	49	76	51	20.28
51-921	X-radiation	Yellow-green	98	80	9,474	708	7.5	168	80	48	88	52	27.43
51-922	X-radiation	Pale green	97	68	8,591	489	5.7	135	68	50	67	50	16.13
51-925	X-radiation	Yellow-green	99	81	8,707	466	5.4	177	88	50	89	50	22.88
50-996	A-bomb radiation	White	95	21	3,069	234	7.6	44	29	66	15	34	0.02
51-917	A-bomb radiation	White	98	92	11,150	713	6.4	186	92	50	94	50	24.77
51-918	A-bomb radiation	White	94	98	12,480	382	3.1	175	98	56	77	44	8.90
51-919	A-bomb radiation	Yellow-green	98	129	13,709	630	4.6	281	129	46	152	54	54.60
51-920	A-bomb radiation	Yellow	99	218	27,491	2,135	7.8	365	218	60	147	40	7.95
51-926	A-bomb radiation	Dwarf white	91	126	15,695	960	6.1	234	126	54	108	46	17.31
51-927	A-bomb radiation	Yellow	96	93	12,436	378	3.0	177	93	53	84	47	15.89
51-929	A-bomb radiation	Cream	90	105	9,558	677	7.1	195	105	54	90	46	28.85
51-930	A-bomb radiation	White	94	94	11,539	983	8.5	166	94	57	72	43	7.86

χ^2 for testing 2 heterozygous:1 homozygous normal plants.

are similar to the parent plants in that they produce a low frequency of mutants.

Discussion

All the mutant lines segregating a distinctly low frequency of recessives reported in this paper were normally fertile and had normal germinability of seeds. Therefore, aberration of male or female gametes and low germination of the mutant seeds probably cannot account for the deviation from the 3:1 ratio. The testcross data of the two mutant lines (Table II) revealed that the low frequencies of mutants were due to reduced transmission of the mutant gene through male gametes. Other lines in which testcross data were not available may have a reduced transmission of the mutant gene in male, or female, or in both kinds of gametes.

Partial elimination of a certain class of gametes resulting in low mutant frequency transmission, has been reported in several mutants of barley.[1, 2, 3] It was suggested in one of these reports[3] that the mutated locus may be the result of, or closely associated with, undetectable chromosomal aberrations such as minute deficiencies or duplications. Similarly the mutants under study in this paper might be due to such aberrations since no detectable aberrations were found. Gametes containing an aberrant chromosome complement could not compete with those containing a normal chromosomal complement during fertilization. This, in turn, would lead to the reduced frequency of gene transmission.

Summary

Seventeen mutant lines of barley, nine induced by atomic-bomb radiation and eight by X-radiation, characterized by a distinctly low frequency of mutant transmission (less than 10 percent) in a selfed population, are reported in this paper. All these lines had normal appearing pollen and normal ovule fertility and seed germinability. Testcross data from two mutant lines showed that only about 10 percent of the male gametes carried the mutant gene instead of the normally expected 50 percent. It is suggested that the mutated loci may be the result of, or closely associated with, chromosomal aberrations, such as minute deficien-

TABLE II. Genetic results of testcross plants

Mutant line		Compana ♂ × Mutant line ♂: Plants producing mutants	Compana ♂ × Mutant line ♂: Plants not producing mutants	Mutant line ♂ × Compana ♀: Plants producing mutants	Mutant line ♂ × Compana ♀: Plants not producing mutants
51-915	No.	30	42	13	80
	Percent	42	58	14	86
51-917	No.	59	50	7	63
	Percent	54	46	10	90

cies or duplications. Male gametes containing such aberrations presumably could not compete with those containing a normal chromosome complement during fertilization. Thus the mutant would be transmitted at a reduced frequency.

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NORMAL INTERCHROMOSOMAL CROSSOVER RELATIONSHIPS

In Drosophilla Melanogaster

H. H. KRAMER AND E. B. LEWIS*

THE assumption that events leading to recombination in a single chromosome pair are independent of similar events in other chromosomes was tested extensively by Muller[4] with 22 mutant genes in chromosomes I and II. He found that the frequency of simultaneous crossovers in the two chromosomes was close to that expected. On the other hand Sturtevant[6] discovered that a chromosome later shown to include an inversion did in fact cause an increase in crossing-over in another chromosome. Since that time convincing data have been presented that inversions in one chromosome increase crossing-over in others. This evidence has been fully reviewed by Schultz and Redfield.[5]

If these data are a reflection of some form of competition for a fixed maximum number of tetrad exchanges per meiocyte as Mather[3] suggested, or if they reflect interchromosomal interference in the synaptic process influenced in some way by heterochromatin as proposed by Schultz and Redfield,[5] similar negative interchromosomal correlations should be apparent in the recombination observed from normal chromosomes. Such data were presented by Schultz and Redfield[5] and interpreted as indicating a negative interchromosomal crossover relationship. The number of regions tested, however, was small and some inconsistencies were apparent.

The experiment reported here was conducted in the hope that by detecting a large proportion of crossovers in all three of the large chromosomes of *D. melanogaster*, a more adequate measure of interchromosomal effects in the standard diploid could be obtained. The experiment is one in which simultaneous segregation for 22 genes marking a large proportion of chromosomes I, II, and III was studied. To minimize viability complications a method of progeny testing was adopted.

The Method

Except for three chromosome rearrangements described below, all of the characters used in this study have been described by Bridges and Brehme.[1] The mating plan to produce females multiply heterozygous for 22 gene loci and to identify the crossover strands from these females is presented in Figure 13. To evaluate heterogeneity it was desired that such females be isogenic for the wild type chromosomes. To aid in producing them a stock with a 6-break chromosome II rearrangement *SM1*† carrying al^2 Cy sp^2 and a 5-break chromosome III rearrangement Ubx^{130} described by Lewis[2] carrying e^s and balanced with *T(2;3) Xa* was used. Canton-S females were mass mated to the balanced males and *Xa* males selected and mated individually to females heterozygous for the three rearrangements *FM1*,‡ *SM1*, and Ubx^{130} marked by *B*, *Cy*, and *Ubx* respectively. All *B;Cy:Ubx* females from this cross thus are isogenic for the Canton-S chro-

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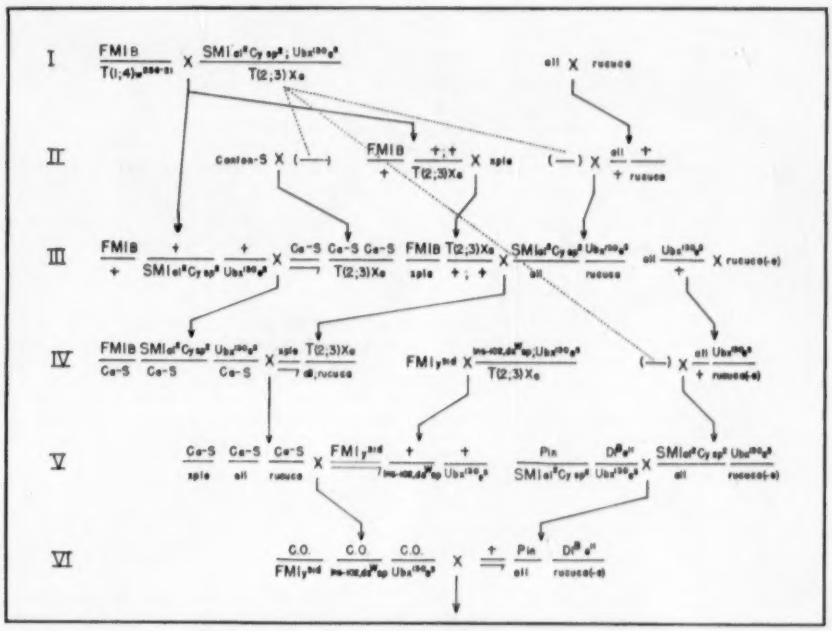
†The new arrangement in *SM 1* is: 2L tip to 22A3/60B6 to 59B1/42A3 to 58A/42A2 to centromere to 34A1/22D2 to 33F/22D1 to 22A3/60C1-2 to tip of 2R.

‡*FM 1* designates *Ins* sc^8 dl^{49} carrying y^{1+t4} $w^a lz^s$ *B*.



MATING PLAN FOR ONE CANTON-S LINE

Figure 13

The mating plan to produce females multiply heterozygous for 22 gene loci and to identify crossover strands in gametes from these females is illustrated graphically.

mosomes. The use of such females increased the chances for non-disjunction of X chromosomes but progenies of females with an extra Y chromosome were discarded in these studies.

The recessive characters were introduced in the following manner: F_1 males from the cross *all* (*al dp b pr c px sp*) × *rucuca* (*ru h th st cu sr e^{s} ca*) were mated to females from the balanced stock and *al sp; e^{s}* males selected and mated to *FM 1,B/Xple; Xa/+* females from the cross of *FM 1,B/+; +/Xa* females × *Xple* (*sc ec cv ct v g f*) males. From this cross *Xple;Xa* males were selected and mated with females heterozygous for the three rearrangements and the Canton-S chromosomes (Generation IV, Figure 13). Wild-type females from this cross were multiply heterozygous. Crossover strands from such females were kept intact by the introduction of the rearranged chromosomes *FM 1,y^{31d}*, *Ins 102,ds^{W}sp**; and *Ubx^{130}e^{s}* so that in the sixth generation each *ds^{W}, Ubx* female contained one gamete resulting from free recombination with each chromosome balanced by a crossover suppressor. The crossover strands were finally uncovered and classified in the progeny of the cross shown in generation VI of Figure 13. In such a progeny each chromosome could be classified independently to avoid epistatic effects. Chromosome I strands could be classified in non-y males which carried the dominants *Pin* and/or *ds^{W}* plus *Dl^{B}* and/or *Ubx*. Second chromosome strands were classified in females carrying neither *Pin* nor *ds^{W}* but having *Ubx* and/or *Dl^{B}*. Similarly chromosome

*The new arrangement in *Ins 102* is: 2L tip to 26A/51C to 41/57A to 51C/26A to centromere to 41/57A to tip of 2R.

III strands were scored in the absence of *Dl*^{B} and *Ubx* but in the presence of *Pin* and/or *ds*^{W}. Classification for *e*^{s} was made separately in flies carrying *Pin* and *Dl*^{B}*e*^{11} since the recessive third chromosome introduced from males in the final cross lacked *e*^{s} (designated rucuca(-e) in Figure 13).

The use of multiple rearrangements as in this study proved to be feasible. Both *FM 1* and *SM 1* are excellent crossover suppressors. The *Ins 102* rearrangement complex was not as effective as *SM 1* particularly in the distal regions but had to be used in the final crosses because the presence of *Cy* in *SM 1* interferes with classification of *cu* in chromosome III. *Ubx*^{130} was found not to be very effective in the *e*^{s}-*ca* region. Crossovers permitted by the rearrangements were sufficiently infrequent, however, that it was deemed sufficient for each chromosome scored to have two flies in each progeny which were phenotypically identical.

Temperatures for matings in the fourth and fifth generations were maintained at 25°C in an incubator, and in the fifth generation, transfer of cultures at 48 hour intervals permitted control of the effect of age of females on crossing-over.

Experimental Results

Approximately 1900 *ds*^{W}; *Ubx*^{130} females in the progenies from 41 multiply heterozygous females representing six Canton-S male lines were testcrossed as shown in generation VI of Figure 13 and classified. Approximately 13 percent of these matings either failed to produce sufficient offspring or the females used were found to have been misclassified for *ds*^{W} or *Ubx*. Of the 1650 successful matings, all of the progenies could be classified for chromosome III strands, but about 3 percent could not be classified for Chromosome I and about 45 percent could not be classified for Chromosome II. Thus there were 854 progenies, in which all three crossover strands recovered from a gamete could be specified.

Chi-square tests for homogeneity among the 41 progenies failed to reveal differences with respect to the percentage of gametes which could be classified for all strands, with respect to percentage of completely classified gametes carrying each of the 22 recessive genes, and with respect to recombination percentages by regions. Consequently all data are presented without regard to parental pedigrees.

A comparison of recovered non-crossover strands revealed that for chromosomes I, II, and III respectively 44 percent (182/410), 5 percent (11/211), and 43 percent (106/244) were multiple recessive strands. These data suggest a relationship between progeny size and the probability of recovering multiple recessive phenotypes. The percentage of testcross progenies showing each of 22 recessive marker genes is given in Table I. In the case of chromosome II, it is apparent that the factor(s) for reduced viability are localized in or near the *pr-c* region. Although the *all* stock used had been tested and found not to contain complete lethals, the difficulty of establishing the stock after testing for lethals was noted.

A summary of recombination by regions in each of the three chromosomes is presented in Table II in comparison with standard map units given by Bridges and Brehme[1]. It may be noted from this table that the agreement of observed values with standard map units is generally excellent even for chromosome II. In the long *dp-b* region some reduction in observed values may be expected. These data indicate that in spite of a failure to recover a large portion of strands carrying *pr* or *c* the strands recovered are representative with respect to the number and positions of crossovers.

In Table III are the gametic frequencies specified completely with respect to the number of crossover points in the recovered strands of each of the three chromosomes. For example, 32 gametes contained non-crossover strands of all three chromosomes while two gametes had a single C.O. strand of I, a triple C.O. strand of II, and a double C.O. strand of III. Expected frequencies for tests of independence were calculated from the totals for each chromosome and are presented in the last part of Table III. It may be noted that there is generally good agreement between observed and expected values which are based on the hypothesis of independence. Results of chi-square tests for independence are presented in Table IV. The probability of an association between the number of crossover points in chromosome I and those in chromosome II approaches the 5 percent level. An inspection of the data in Table III reveals, however, that the association tends toward a positive one *i.e.* few recombination points in

TABLE I. Percentage of 854 testcross progenies showing indicated recessive gene

Chromsome I		Chromsome II		Chromsome III	
Gene	%	Gene	%	Gene	%
sc	43.6	*al*	39.5	*ru*	49.5
ec	43.4	*dp*	34.5	*h*	46.8
cv	44.1	*b*	28.9	*th*	47.4
ct	44.8	*pr*	22.0	*st*	47.9
v	47.3	*c*	20.3	*cu*	46.5
g	48.9	*px*	35.9	*sr*	46.6
f	51.2	*sp*	40.4	*e*	46.4
				ca	43.3

I associated with few in II. The probability of association between chromosomes II and III approaches the 10 percent level and again the trend, if a trend may be said to exist, is in the direction of a positive association.

Discussion

The data reported herein appear to demonstrate effectively that, insofar as the strands recovered from prophase I tetrads in oöcytes of *D. melanogaster* are concerned, there is little or no interchromosomal crossover effect. To the extent that strands are drawn randomly from each tetrad, a similar conclusion might be made regarding the interchromosomal relations of tetrad exchange points. Numbers of crossover points in recovered strands, however, can not be regarded as a sensitive measure of numbers of exchange points in tetrads since a non-crossover strand does not imply a no-exchange tetrad. In tetrads with a given number of exchange points, the recovered strands are distributed binomially with the probability of recovering a non-crossover strand being equal to that of recovering a strand with as many crossover points as the number of exchange points in the tetrad. This relationship is shown in Table V. Thus, while the regression of average crossover points per recovered strand on number of exchange points per tetrad is 0.5, the sampling deviations may be rather wide as indicated by the high standard deviations.

A second factor which may reduce the correlation between tetrads and recovered strands is the possibility of oögonial crossing-over[7]. One case which could be interpreted by assuming such a crossover was found in the progeny of female 5-2 in which all of 28 tested offspring

TABLE II. Percent recombination by regions from 854 gametes compared with standard values

Chromosome I			Chromosome II			Chromosome III		
	% Recombination			% Recombination			% Recombination	
Region	Observed	Standard	Region	Observed	Standard	Region	Observed	Standard
ec-ec	4.3	5.5	*al-dp*	15.0	13.0	*ru-h*	24.1	26.5
ec-cv	9.1	8.2	*dp-b*	30.3	35.5	*h-th*	19.8	16.7
cv-ct	7.0	6.3	*b-pr*	7.7	6.0	*th-st*	0.7	0.8
ct-v	15.3	13.0	*pr-c*	19.9	21.0	*st-cu*	5.0	6.0
v-g	11.0	11.4	*c-px*	22.6	25.0	*cu-sr*	12.6	12.0
g-f	11.1	12.3	*px-sp*	7.5	6.5	*sr-e*	9.3	8.7
						e-ca	31.0	30.0
Sum	57.8	56.7		103.0	107.0		102.5	100.7

TABLE III. Summary of crossover strands of three chromosomes of *D. melanogaster* in 854 gametes

No. of Crossovers in II	Observed No. of Crossovers in III					Expected No. of Crossovers in III				
	0	1	2	3	Sum	0	1	2	3	Sum
					Non-crossovers in I					
0	32	47	18	6‡	103	29.0	45.5	22.3	4.5	101.3
1	63	84	54	11	212	59.5	93.7	45.9	9.3	208.4
2	20	19	22	6	87	25.0	39.3	19.2	3.9	87.4
3	3†	4	1	0	8	3.7	5.8	2.8	0.6	12.9
Sum	118	174	95	23	410	117.2	184.3	90.2	18.3	410.0
					Single crossovers in I					
0	24	47	19	6	96	27.8	43.8	21.4	4.3	97.3
1	63	91	42	5	201	57.2	90.0	44.1	8.9	200.2
2	18	36	22	2‡	78	24.0	37.8	18.5	3.7	84.0
3	5	12	2	0	19	3.6	5.6	2.7	0.6	12.5
Sum	110	186	85	13	394	112.6	177.2	86.7	17.5	394.9
					Double crossovers in I					
0	2	8	2	0	12	3.5	5.6	2.7	0.6	12.4
1	11*	7	3	0	21	7.3	11.4	5.6	1.1	25.4
2	3	9	3	2	17	3.0	4.8	2.4	0.4	10.6
3	0	0	0	0	0	0.4	0.7	0.4	0.1	1.6
Sum	16	24	8	2	50	14.2	22.5	11.1	2.2	50.0
					Total in I					
0	58	102	39	12	211	60.3	94.9	46.4	9.4	211.0
1	137	182	99	16	434	124.0	195.1	95.6	19.3	434.0
2	41	84	47	10	182	52.0	81.9	40.1	8.0	182.0
3	8	16	3	0	27	7.7	12.1	5.9	1.3	27.0
Sum	244	384	188	38	854	244.0	384.0	188.0	38.0	854.0

*Includes 1 triple crossover in I
†Includes 1 quadruple crossover in II
‡Includes 1 quadruple crossover in III

showed that they had received *ru* and *h* but had normal recombination for the remainder of chromosome III and for the other two chromosomes. In this case, however, the possibility of a new lethal mutation in the wild-type chromosome in or near the *ru-h* region is not excluded.

A third factor is the effect of age of females on recombination. Since cultures were transferred at 48-hour intervals, age effects could be determined and are presented in Table VI. Although numbers are too small to follow changes by separate regions, the usual trend of reduction during the first six to eight days is shown by chromosomes II and III. Simultaneously, however, recombination in the region *sc-f* in chromosome I is increasing. These trends should result in a shift toward a positive association for chromosomes II *vs* III which, although observed, was not significant. Comparisons of chromosome I with II and III should show a shift toward a negative association but in this case a slight but non-significant positive trend was noted. It would appear that age of female can not be regarded as having an important effect on the relationships observed here.

Although no evidence of interchromosomal crossover effects was found the data reported herein do not necessarily rule out the possibility of negative chromosomal crossover relationships. On the other hand, the negative relationships obtained through the use of heterologous inversions do not permit an unqualified extension to the normal case.

TABLE IV. Chi-square analysis for association of crossover strands in 854 gametes of *D. melanogaster*

Source of Variation	Degrees of Freedom	χ^2	P
Chrom. I vs. II	6	12.01	.05
Chrom. I vs. III	6	4.97	.50
Chrom II vs. III	9	13.51	.10
Chrom. I vs. II vs. III	18	15.07	.50
Total	39	45.56	.40

TABLE V. Relation of exchange points per tetrad and number of cross-over points per recovered strand

Points per tetrad	Points per recovered strand	
	Mean	S.D.
0	0.0	0.00
1	0.5	0.50
2	1.0	0.71
3	1.5	0.87
4	2.0	1.00
5	2.5	1.12

TABLE VI. Total Recombination by two-day periods

Days	No. of Gametes	Chromosome I	II	III
1-2	185	54.6	107.0	117.3
3-4	317	55.2	106.0	100.9
5-6	227	61.7	99.1	98.2
7-8	80	61.2	95.0	93.8
9-10	45	66.7	100.0	91.1
Sum	854			
Ave.		57.8	103.0	102.5

Summary

By a system of matings and progeny tests in *Drosophila melanogaster* whereby chromosomes I, II, and II could be classified independently, and with minimal viability effects, 854 complete gametes from females heterozygous for *sc ec cv ct v g f* on chromosome I, *al dp b pr c px sp* on chromosome II, and *ru h th st cu sr e' ca* on chromosome III were analyzed.

It was found that no association between the number of crossover points on one chromosome and the number of such points on other chromosomes could be demonstrated.

It is suggested that on the basis of data so far accumulated, an unqualified extension to the normal case of the negative interchromosomal crossover relationships obtained through the use of heterologous inversions may not be completely warranted.

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WOOLLY FEATHERING IN THE FOWL

D. G. Jones and Walter Morgan*

TYPICAL WOOLLY CHICK

Figure 14

The appearance of abnormal "rolled feather nodules," particularly in the dorsal pteryla, of a newly hatched woolly chick, is shown.

DURING the course of breeding experiments which involved the mating of near relatives, a new abnormal feather mutation was discovered in the White Plymouth Rock stock at this station in 1948. In a preliminary report, Jones and Kohlmeyer[4] briefly indicated how the mutant birds looked and told of the stock in which it was found. A description of the mutant phenotype, segregation ratios and its effect upon reproductive performance are reported herewith.

Description

At hatching time the chicks with the abnormal down show considerable variation in the completeness of feathering. The down covering on most chicks is intermediate in completeness between the normal and a semi-naked condition. A few have relatively little normal appearing down while some others appear normal except for a pronounced shortening of the down.

The chicks which are most devoid of down lack all of the down normally present in the apteria of the dorsal and abdominal feather tracts. Also, individual down feathers in the adjacent pterylae of the dorsal, femoral, humeral and abdominal feather tracts are sometimes rolled into the shape of small feather nodules. The occurrence of these rolled nodules in conjunction with the absence of down in the apteria gives the more extremely affected chicks a semi-naked appearance. In the less severely affected chicks the length of the down in the mutants is much shorter than for the normals and rolled nodules occur infrequently. However, a comparatively

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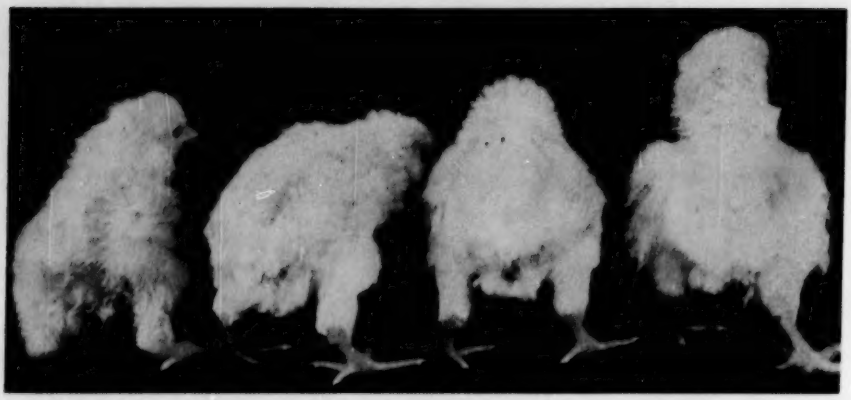


SCRAGGLY CHICKS

Figure 15

Four newly hatched woolly chicks showing their typical scraggly appearance.

easy criterion for positively identifying mutants at hatching time has been the slick, shiny appearance of the feet and legs, particularly the backs of the shanks and bottoms of the feet.

Juvenile and adult feathers are normal in quantity and distribution, but individual feathers are defective. Grossly, the pterylae appear normal but the feathers lack the smooth, well groomed appearance which is associated with normals. They are rough and incomplete rather than smooth and contributing to an even, neat symmetry. There is an imperfect interlocking of barbules. As a result, the barbs are not held together. Microscopically, many of the barbules from abnormal chicks lie almost parallel to the barbs, instead of in the normal perpendicular position, and often the barbules are missing. In addition, many of the hooklets are missing and those which are present appear abnormal.

Genetics

When the first birds having abnormal plumage reached maturity, they were mated with normals in an effort to determine the mode of inheritance of the mutation. Matings #1 and #2 in Table I represent cumulative data. Inasmuch as no woollies ever appeared in the F_1 it was apparent that a recessive mutation was involved. Also, it was obvious that a sex-linked gene was not concerned, since reciprocal matings between woollies and normals produced only normal chicks.

Subsequent F_2 matings (#3 in Table I) produced 433 individuals which included newly hatched chicks and embryos which survived to the eighteenth day. The ratio of 325 normals to 108 woollies fits the expected F_2 ratio of a simple Mendelian recessive very well; $\chi^2_{(1)} = .0008$, $P = .95 - .98$. Matings of heterozygotes with homozygous normal birds (#4 and #5) provided additional data proving that the woolly gene behaves as a complete recessive.

A testcross ratio of 40 normals to 43 woollies again indicated normal segre-

TABLE I. Phenotypic ratios from matings involving the woolly gene. (1948 & 1956)

Mating	Parental types		Progeny Phenotypes	
	Sire	Dam	Normal	Woolly
#1.	Normal	Woolly	43	0
#2.	Woolly	Normal	233	0
#3.	F_1 Normal	F_1 Normal	325	108
#4.	F_1 Normal	Normal	671	0
#5.	Normal	F_1 Normal	272	0
#6.*	Woolly	F_1 Normal	40	43
#7.	Woolly	Woolly	0	95

*Includes all chicks developing to 18 days of age, whether hatched or dead-in-shell.

gation of the mutant gene. Moreover, the final mating type of two woolly homozygotes (#7) resulted in only woollies thus indicating complete penetrance with no overlapping of normal phenotypes.

Comparative Reproductive Performances

Weak appearance of woolly chicks at hatching time suggested that a deleterious effect might be manifested in the developing embryo. In order to study the possible reduction in parental fertility of birds carrying the woolly gene or in hatchability of embryos which were heterozygous for the woolly gene, which we shall call *wo*, Table II was constructed. Although the *wo/wo* sire genotype gave a lower fertility than *Wo/wo*, the hatchability of the former mating was approximately the same as the latter. Under normal circumstances it might be expected that the F_2 hatchability, because of additional heterogeneity, would also be higher. However, late death of the *wo/wo* embryos, as will be discussed shortly, served to counterbalance this advantage. It was not too surprising to find that fertility and hatchability percentages for both groups compared favorably with averages from stocks not involving *wo* because the good fit of the F_2 and testcross ratios indicated that the *wo/wo* embryos were not quantitatively deficient and therefore an early lethal effect was unlikely.

A disproportionate number of woolly chicks was found dead in the shell during the last three days of incubation. The mortality comparisons in Table III show that approximately twice as many woollies died late as did the normals. Although a progressively increasing death incidence is observed for the three mating types in Table III, the combined data are from different years and environmental or seasonal variables may account for the fluctuation. The important fact is that 23.1 percent is approximately twice as much as 11.4 percent and 39.5 percent is approximately twice as much as 20.0 percent; both advantages being in favor of the non-woollies. There was some variation within matings and it is evident that in those matings where the general level of hatchability was high, there seems to be little evidence that this mutation is a serious handicap to the affected chick with respect to hatchability. It seems probable that if genes for high hatchability in general are present, this mutation does not seriously affect the hatching ability of the eggs but if such genes are not present, hatchability is depressed.

TABLE II. Effect of the woolly gene upon fertility and hatchability. (1955 & 1956)

Sire	Dam	Eggs Set	Fertiles	% fert.	Chicks	% hatch of fert. eggs
Normal	Normal	1780	1627	91.4	1422	87.4
Woolly	Normal	197	175	88.8	154	88.0
F_1 Normal	F_1 Normal	214	204	95.3	177	86.8

WING FEATHERS

Figure 16

The main wing feathers of a white woolly chick are shown at four weeks of age.

Post-hatching Influences

In order to compare livability of woollies with normals, for the first two weeks, the chicks were kept in batteries in comparable age groups, with no preference being given to either phenotype within the intermingled populations. Woolly chicks appeared to be less active immediately after hatching and consequently seemed to be "pushed around" con-

TABLE III. Effect of the woolly gene upon late embryonic death. (1948 & 1956)

		Normal			Woolly		
		Number of	Dead at 19-21 days		Number of	Dead at 19-21 days	
Sire	Dam	Chicks Hatched	Number	Percent*	Chicks Hatched	Number	Percent*
Woolly	Normal	154	9	5.5	0	0	0.0
F_1 Normal	F_1 Normal	288	37	11.4	83	25	23.1
Woolly	F_1 Normal	32	8	20.0	26	17	39.5

*Of embryos alive at 19 days.

BARRED WOOLLY

Figure 17

A two week old woolly chick showing the barred pattern.

siderably. Inasmuch as the woollies stayed near the heat source for much of the time, it was apparent that they required a greater amount of environmental heat than did the normals. Therefore it was not surprising that more mutants died during the first two weeks than did normals. Among normal and woolly chicks (104 of each kind) mortality to two weeks of age was 5.8 and 12.5 percent, respectively. Actually, the surprising fact was that those scraggly looking chicks had a two week survival rate as high as 87.5 percent.

The effect of the woolly feathering was perhaps most disadvantageous during the range rearing period. Under range conditions the affected birds were very susceptible to sudden showers since the defective feathers did not shed water normally and soon became thoroughly wet. Subsequent chilling often resulted in death.

Two *wo* genes in one bird did not appear to affect the sexual maturity of males if comb development can be used as a criterion. Development of the secondary sexual characteristics in woolly males occurs at approximately the same age as in normals. Since development of the comb occurs just prior to the onset of spermatogenesis, it seems probable that woolly males become sexually mature at approximately the same age as do the normals. However, the woolly pullets appeared to reach sexual maturity (as measured by date of first egg) later than the normals.

Adult woollies appeared to be smaller than the normal birds. This apparent smallness was due not only to the fact that the abnormal feathers fit more closely to the bird's body than did feathers of the normals, but also to a decided difference in body weight (Table IV). In the relatively small sample of mutants which were weighed, the adult body size was effectively limited in both sexes when birds were homozygous for the *wo* gene. Closely accompanying the reduction of body size was a reduction in egg size to below the average attained by normal females of the same strain.

Discussion

Several of the reported feather mutations have abnormalities in common with woolly. For instance, the "clubbed" down reported by Lepkovsky *et al*[5] is similar to the "rolled feather nodules" observed in many woolly chicks. Severely affected woolly chicks were also somewhat similar to the naked chicks described by Hutt and Sturkie[2]. The ropy chicks described by Warren[7] and the stringy chicks of Buss *et al*[1] look like some of the woolly mutants because they have in common a failure of the down to "fluff out." However, no adult woolly feathers were observed to have the typical fissure in the shaft found in ropy birds or the short adult feathers as found in stringy mutants.

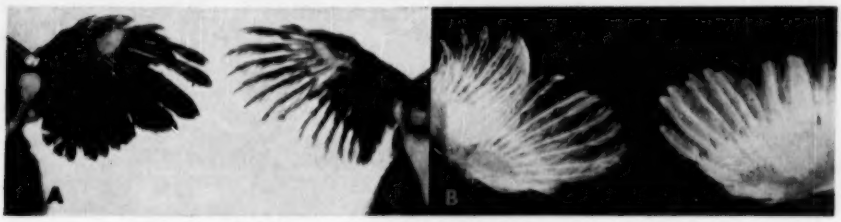
Upon gross examination, the defective woolly feathers appeared much like those described by Warren[6] as "frayed." However, one difference is that with the woolly birds all feathers are abnormal rather than just the large feathers of the wings and tail.

Although the plumage of the woolly birds has a ragged appearance, there seems to be no relation to this mutation and "ragged wing" which was reported by Hutt *et al*.[3] Tail feathers and wing feathers of woollies are not shortened.

The woolly gene fits into the same category as many of our morphological recessives. To date we have not discovered any beneficial effect resulting from the presence of this gene in heterozygotes or homozygotes. To the contrary, there are harmful reproductive effects manifested in the *wo/wo* birds. This means that the poultryman would desire to eliminate the gene from his population so that his birds would perform at their maximum level. It is difficult to eliminate a recessive gene from birds which are all phenotypically normal, particularly in a mass mating. Birds which are homozygous for the mutant thus provide valuable material for two purposes: (1) genetic and physiological studies of linkage and gene action, and (2) tester birds

TABLE IV. Average adult body weights of woollies and normals (1948)

Phenotype	Sample-size		Weight in pounds	
	Males	Females	Males	Females
Normal	18	401	7.9	5.4
Woolly	5	9	6.0	3.8



WING FEATHERS OF BARRED AND WHITE CHICKS

Figure 18

A— Wing feathers of two week old barred chicks. Normal on the left, woolly on the right. *B*—Wing feathers of four week old white chicks; woolly on the left, normal on the right.

which can help eliminate carrier heterozygotes when used in testcross matings.

Summary

A feather mutation in the fowl which can be recognized in newly hatched chicks as well as in the adult feathers is described. The symbol *wo* is suggested for this mutation which results in a woolly appearing homozygote. The gene behaves as a simple Mendelian recessive with complete penetrance. An absence of overlapping, in the test stocks, suggests that *wo* would be a good marker gene to be used in linkage studies. Effects of the gene on reproduction and survival are discussed.

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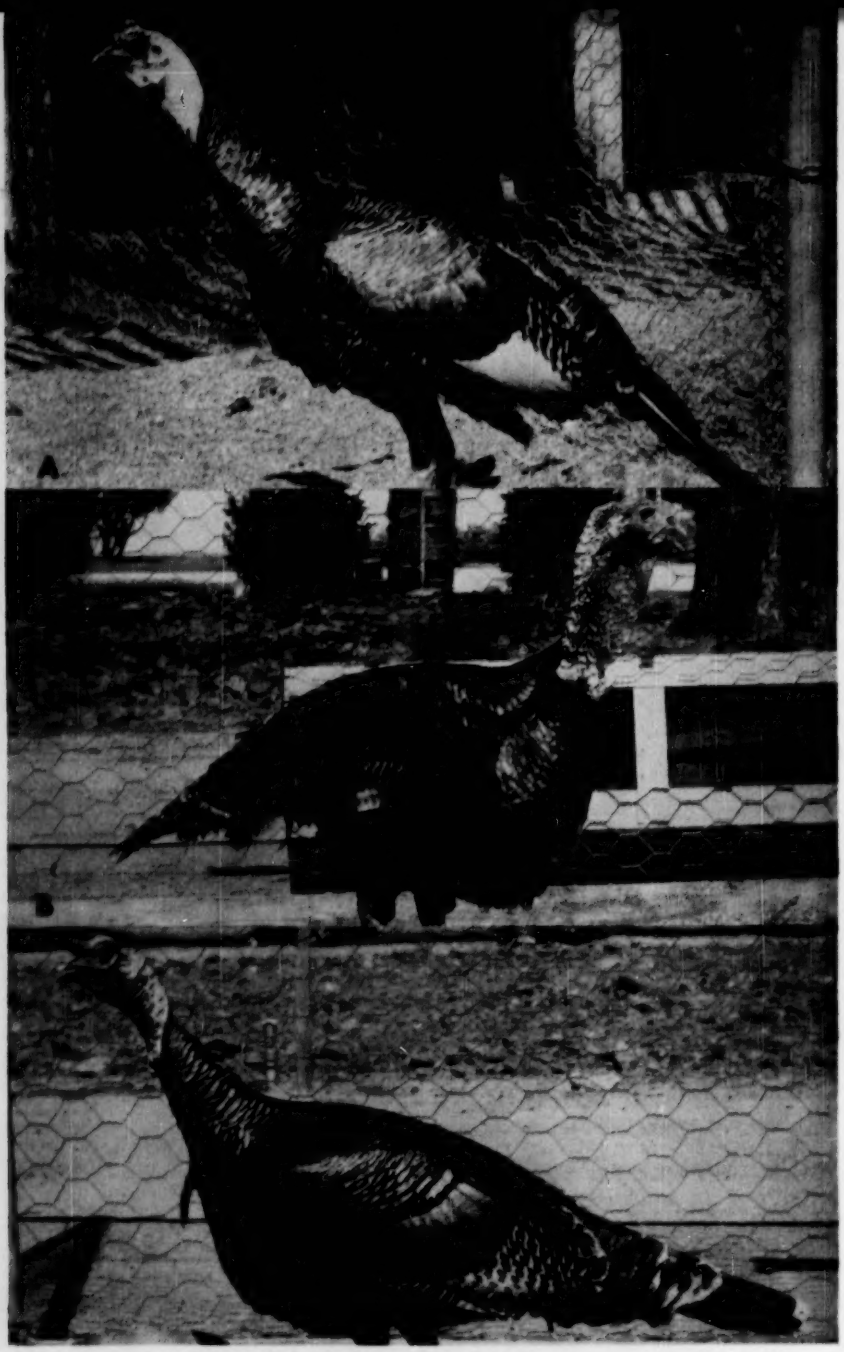


ADULT MALES OF THE PARENTAL SPECIES AND F_1

Figure 19

At the time these pictures were taken (late March 1956) the ocellated bird (*A*) was just coming into breeding condition and the head furnishings were not completely developed. The domestic bronze (*B*) by contrast had been in breeding condition for several months. Note the fully developed head furnishings and the ragged feathers characteristic of domestic turkeys late in the breeding season. The F_1 (*C*) is intermediate in size and has head furnishings intermediate in condition and appearance between the parental species. The snood, miniature in the *ocellata*, is well developed but the fleshy knob on the head of this parent is absent from the F_1. The F_1 also possesses a well developed brush, characteristic of the bronze but absent in *ocellata*.

TURKEY HYBRIDS

(Meleagris ocellata × *Meleagris gallopavo)*

F. W. Lorenz, V. S. Asmundson and N. E. Wilson*

MELEAGRIS gallopavo was introduced into Europe at the time of the conquest of Mexico early in the sixteenth century.[14] Its ready acceptance as a domesticated bird and rapid spread throughout the continent are evidence of its prior domestication in Mexico. This bird is generally believed to be derived from *M. gallopavo gallopavo.*

The ocellated turkey (*Meleagris ocellata*), by contrast, has apparently never been domesticated. This bird is restricted in natural habitat to tropical lowland forests in Guatemala, British Honduras, and Yucatan, where it is quite rare; in captivity it is exceedingly wild and difficult to propagate.[8] It is usually placed in the genus *Meleagris* but is regarded as a species distinct from domesticated turkeys and the various wild subspecies of *M. gallopavo.* Chapman[4] proposed placing it in a distinct genus (*Agriocharis*), although Ridgway as quoted in Friedman[5] favors the older classification. Beaks of the fossil *Parapavo californicus* (Miller) resemble those of *Meleagris ocellata* more than do those of *M. gallopavo*,[6,7] Sushkin[12] on the basis of a study of leg bones and the coracoid, suggested that the difference between the Pleistocene *P. californicus* and *M. ocellata* "may not even be sufficient to warrant their generic separation." It is of interest in considering the relationships of these various species that bones of Pleistocoene turkeys in Florida resembled those of *M. gallopavo.*[13]

The *ocellata* is a much smaller bird than the *gallopavo;* the two species differ in plumage color, in the distribution, number, shape, and size of caruncles on the head and neck, and in the size of the snood. The *gallopavo* male has a beard, the *ocellata* none. The voice is also different.[5,8]

In order to obtain data on the fertility of the two species when crossed, arrangements were made to obtain semen by the method of Burrows and Quinn[3] from an ocellated male at San Diego Zoo,[8] which was used to fertilize virgin Broad Breasted Bronze females. The eggs from these hens were shipped to the University of California Experiment Station at Davis and hatched there.

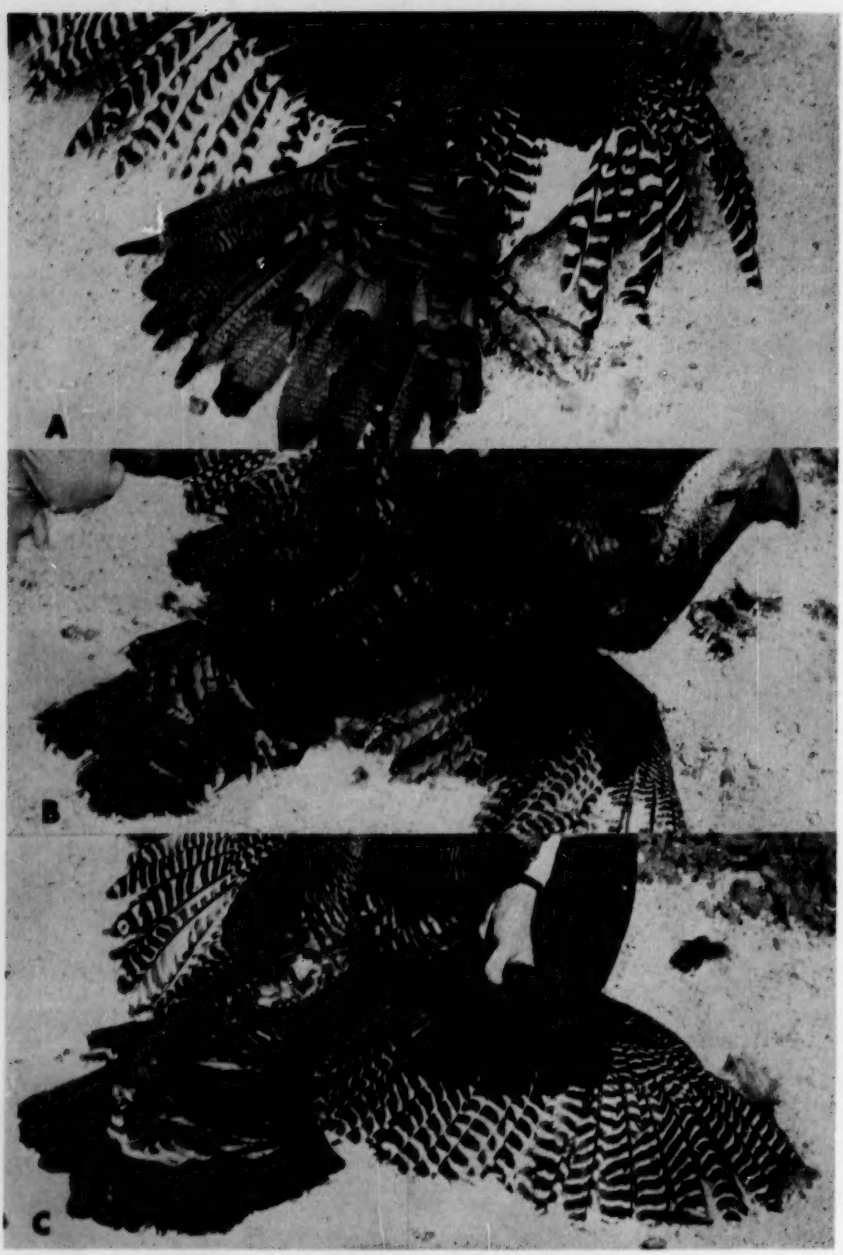
In 1954 the ocellated male of a pair kindly donated to the University of California by the San Diego Zoo† was also successfully crossed by artificial insemination with *gallopavo* (Bronze and other domesticated varieties). This report deals only with the fertility of this cross and of their progeny in backcrosses on Bronze.

Results

The F_1 progeny are intermediate in weight and in plumage color. The plumage color is less brilliant, the ocelli are more subdued, and there is less gray in the plumage of the F_1 than in that of *ocellata.* Body feathers of the *ocellata* possess both green and bronze iridescence, but green predominates. The bronze emerges in the secondary coverts which contrast strikingly with the green body. In the F_1 this distribution is reversed; green is more prominent in the wing bar than in the body feathers, where it is limited to the posterior regions and is relatively restricted even there. Thus, the F_1 is predominantly bronze with a green bar in contrast to the green body with bronze bar of the

*Poultry Department, University of California, Davis. We are greatly indebted to Mrs. Belle Benchley, Executive Secretary, and Mr. K. C. Lint, Curator of Birds, both of the San Diego Zoological Society, and to the Society for gifts of birds; to Mr. J. R. Carson, who furnished the hens for the original crosses; and to Mr. Robert H. Adolph, Farm Advisor, who handled local arrangements for the care of the birds and shipment of eggs.

†Two pairs donated previously by the San Diego Zoo failed to survive long enough to leave progeny.



PLUMAGE PATTERNS

Figure 20

The same birds shown in Figure 19 are held spread out to display plumage patterns. Note the reduced ocelli and the coarser grey stippling in the tail feathers of the F_1 (*C*) as compared to the *ocellata* (*A*) and the white tipping of the tail coverts which is characteristic of the bronze (*B*).

ocellata. The bronze iridescence of the F_1 is darker than that of the *ocellata* but much lighter and brighter than that of the domestic turkey. Standing in the sunshine it appears to be covered with burnished copper.

The snood of the F_1 is large, but in other respects the featherless part of the head and neck is intermediate or more like that of the *ocellata*. The F_1 male has a beard, but it is smaller than the beard of domesticated turkey males. The voice of the hybrids is similar to the voice of *ocellata*.

The hatching results are summarized in Table I. The low fertility obtained in 1952 was probably due in part to a late start and the decrease in hatch caused by shipping. Three of the 71 eggs received were broken and one had a moveable air cell which usually causes death of the embryo. No doubt the same conditions that caused breakage also reduced the hatchability of the apparently unaffected fertile eggs. It is apparent that fertility of eggs from this cross was low. In 1954 and 1955 one ocellated male was available at Davis. The meager quantity of semen obtainable from him was divided among several hens in an attempt to obtain the maximum number of progeny. Each hen thus received less than the amount usually found necessary for optimum fertility;[9,10] hence, better fertility would probably have been obtained had an adequate amount of semen been available. As often observed with inadequate dosage, some inseminations were completely unsuccessful in 1954. Thus, if only those settings from individual hens that included some fertile eggs are considered, the fertility for this year would be 46.0 percent, which is similar to the fertility obtained in 1955. The hatch of fertile eggs in 1954 and 1955 was not significantly different from what might be expected from Broad Breasted Bronze turkeys.

The fertility and hatchability data for backcrosses on Bronze are not consistent. In 1953 only two F_1 males were available; they matured late and produced a low volume of semen. In 1955 about 20 F_1 males were on hand; the one chosen for the backcross mating furnished an abundance of semen which was administered to five hens, but the average resulting fertility was only 74.5 percent (Table I). Fertility of the eggs from four of these hens however, was 97 percent. It seems safe to conclude that good fertility may be expected in the backcross if adequate amounts of semen are available. This is also true where backcross hens are used as shown by the last two lines of Table I. Firm conclusions about relative fertility and hatchability of backcrosses must await additional data since only two hens out of the backcross made in 1953 were available.

Sex Ratios

The data available on the sex of the F_1 backcross progeny are submitted in Table II. Data for embryos that survived 20 days are included. The one F_1 hen hatched in 1952 laid 63 eggs in her first year, but all except three were soft-shelled; none hatched although she was inseminated with semen from Bronze males and some of the eggs were fertile. She has continued to lay soft-shelled eggs and no progeny have been obtained from her. The eight known F_1 females hatched and banded in 1954 all died before maturity.

TABLE I. Fertility and hatchability of eggs out of domesticated (*M. gallopavo*) female turkeys mated to ocellated males and backcrossed on domesticated turkeys

Dam	Sire	Year	Eggs set No.	Fertile percent	Dead embryos in percent of fertile			Hatched percent of fertile
					1st test	2nd test	Failed to hatch	
Bronze	Ocellated	1952	71	25.4	28.7	28.7	22.2	22.2
Bronze	Ocellated	1954	244	27.8	19.1	4.4	8.8	67.6
Mixed domestic	Ocellated	1955	64	53.1	23.5		8.8	67.6
Bronze	F_1	1953	71	25.4	55.6		5.6	38.9
Bronze	F_1	1955	94	74.5	4.3		8.6	87.1
Backcross	F_1	1954	24	75.0	33.3	22.2	27.8	16.7
Backcross	Backcross	1954	48	93.8	26.7		37.8	35.6

Nine birds were lost with sex unrecorded, but regardless of the sex of these, it is apparent that of the embryos that survived 20 or more days there were fewer females than males. This was true for all groups except the F_1 progeny in 1955 for which final data are not yet available. Data from other sources indicate that the accuracy of the 1955 sex data should exceed 95 percent, which would mean that they agree better with sex ratios for domesticated turkeys[1] than those for other years.

The viability of the male hybrids has been good, but it is not yet certain that this is also true of the female hybrids. The hybrids are comparatively wild and there is an extreme tendency to fight which has made control of cannibalism and resulting mortality difficult. The backcrosses from F_1 males and domesticated turkeys are more docile and resemble the domesticated birds in size and appearance. The viability of birds hatched from backcrosses has been good.

Discussion

The data so far available for the cross of ocellated male on domesticated (*M. gallopavo*) females indicate: (1) that fair to good fertility can be obtained, (2) that hatch of fertile eggs is comparable to that of eggs from domesticated turkeys, (3) that post-hatching viability, at least of the males, is good and, (4) that the hybrids are fertile. The male hybrids are fully fertile in backcrosses on the domesticated turkeys; the one hybrid hen for which data are available has laid well but has failed to lay eggs with normal shell. Whether or not this behavior is typical of such hens remains to be determined. These results differ from those obtained when different genera, such as turkeys, domestic fowl (*Gallus gallus*), and pheasants (*Phasianus colchicus*) are crossed, for in such intergeneric crosses[2,11] fertility and hatchability were reduced and the hybrids were sterile. The comparatively good fertility obtained when the two species of turkeys are crossed, together with the fertility of their hybrids, suggests that all turkeys belong to the same genus.

Summary

Crosses were made by artificial insemination between *M. ocellata* males and domesticated (*M gallopavo*) females. The F_1 males have been found to be fertile in crosses with domesticated hens. The single F_1 hen laid as well as domesticated hens, but nearly all of the eggs were soft-shelled and none hatched. The two species are thus interfertile, but it is not yet certain whether hybrids equal the species in fertility and viability.

The F_1 progeny are intermediate in all traits observed except the voice, which is similar to that of *ocellata*.

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TABLE II. Sex of hatched birds and embryos that failed to hatch

Dam	Sire	Year	Hatched ♂	Hatched ♀	Hatched ?	Not Hatched ♂	Not Hatched ♀	Not Hatched ?	Total ♂	Total ♀	Total ?
Bronze	Ocellated	1952	2	1	1			4	2	1	5
Bronze	Ocellated	1954	29	8	9	1	4		30	12	9
Mixed domestic	Ocellated	1955	10*	11*			5		10	16	
Bronze	F_1	1953	6	2			1		6	3	
Bronze	F_1	1955	36*	25*		1	5		37	30	
Backcross	F_1	1954	1	2		4	1		5	3	
Backcross	Backcross	1954	10	6		10	7		20	13	

*Sexed by the cloacal examination method.

THE INHERITANCE OF BEHAVIOR

Activity Differences in Five Inbred Mouse Strains

WILLIAM R. THOMPSON*

IN a previous experiment[3] surveying the behavior of 15 mouse strains, the writer found striking differences between some of these strains in activity-level, or, as it was called then, strength of exploratory activity. This trait was measured empirically in terms of the distance traversed by a mouse in an unfamiliar situation during a 10-minute period. Some strains were found to be highly exploratory, covering a considerable distance in the test environment, while other strains hardly moved from the starting-box. As so defined, activity-level is perhaps basic to the behavior of most living forms. In nature, an organism is continually required to face and to investigate novel surroundings, and often, in fact, its survival depends on how quickly and efficiently it can do so. This being so, the importance of activity-level in an unfamiliar environment can hardly be overlooked by anyone interested in animal behavior, and in recent years its popularity as a subject for scientific inquiry has been growing rapidly. Among the many problems that can be studied in connection with activity-level, one of obvious value and interest is the manner in which the trait is inherited and the extent to which it can be altered by environmental factors. The present experiment is an attempt to confirm and extend previous observations on the inheritance of activity-level in a few of the more widely-separated strains of mice, and to establish a ground-work for thorough genetic analysis later.

Materials and Methods

Animals. Five genetically pure mouse strains were studied: C57BR/a, C57BL/6, AKR, BALB/c, A/Jax. The morphological and physiological characteristics of these lines have been described elsewhere.[2] Samples of either 20 or 40 mice (equal numbers of each sex) from each of these strains were observed in two activity tests described below. The animals were between 60 and 90 days of age at the time of testing. Inspection of the obtained data showed that age variation within this range did not bear a significant relation to activity level and could therefore be ignored.

Apparatus. Two devices were used to measure activity-level. Test 1 has previously been described.[3] Briefly, it consisted of a square compartment 30 × 30 × 3¾ in. with a wire mesh top and a floor divided into 25 squares. A single-unit barrier was placed at the base of every other square, and in one corner there was a small starting-box leading into the main testing area. Activity-level was measured in terms of the number of squares traversed in a 10-minute period. Test 2 was a Y-maze with arms 11½ in. long × 3 in. wide × 3½ in. deep. The angles between arms were equal. The stem of the maze was painted gray, the left arm black and the right arm white. The animal was put in at the base of the stem and allowed to explore freely for 10 minutes. A record was taken of the number of half-arm units it traversed during each of the six 100-second periods. In both tests, observations were made under dim and constant illumination, the experimenter being hidden at all times from the view of the animal. The tests each had a reliability in excess of 0.90, and intercorrelated about 0.60.

Results

As shown in Table I, the five strains differ strikingly in activity level as measured by both tests. The means on Test 1 are based partly on samples of animals tested in an earlier experiment.[3] It is noteworthy that the

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addition of more subjects to these original samples did not influence the strain-means significantly, and that the rank-order of strains with respect to activity-level is exactly the same as previously reported. Since the variances of the strains differed considerably, no statistical tests of the differences between the means was attempted. However, it should be mentioned that there was no overlap between C57BR/a mice and A/Jax mice and only one case of overlap between C57BR/a and BALB/c mice.

The results obtained on Test 2 agree closely with the above data. Mean number of units traversed in the Y-maze by the five strains are presented in Table I and their activity as a function of time is represented graphically in Figure 21. It is again evident that the strains vary markedly in activity-level and that they retain the same rank-order as in Test 1 with the exception of C57BL/6 mice which have a mean score in the second test lower than AKR. This may have been due to some unforeseeable disturbances near the testing room at the time. Variances again also differ too much to permit statistical tests to be computed on mean differences. However, it is fairly clear by inspection that they are large, and, in fact, there was no overlap in scores between C57BR/a mice and either BALD/c or A/Jax mice.

The time-course of activity shown by the five mouse-strains is very similar to that obtained with dogs[4] and rats.[1] In all three cases, there occurs a decline of activity with time, the rapidity of this decline being sharper with dogs and rats and more gradual with mice.

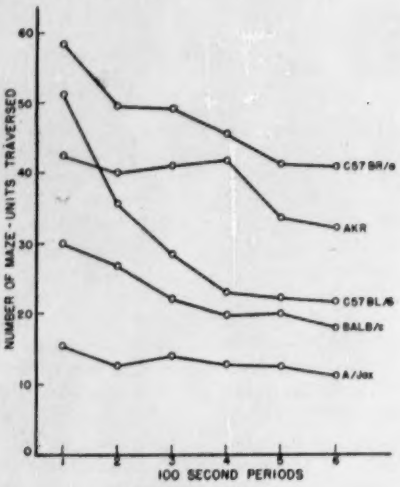
Discussion

On the basis of the two tests, it may be safely concluded that hereditary factors play an important part in determining the activity-level of mice. This is not to say, of course, that environmental effects are negligible. On the contrary, from work with dogs,[4] there is every reason to believe that they can have a marked influence on activity. In the present study, however, such effects were probably of no importance, since all animals were reared and tested under ostensibly identical conditions; and, although the variations within strains that have been bred for genetic homogeneity may appear large, they are probably small compared to the variability of animals from a random stock. This is not altogether conjecture. As a rough test, four mice from a colony of *Peromyscus maniculatus bairdi* being raised by Dr. John A. King of the Jackson Laboratory were run in Test 1. These animals showed a range in score from 23 to about 2000 squares traversed in 10 minutes, a difference far larger than that between the most active C57BR/a and the least active A/Jax. In view of this comparison, we may regard the within-strain variances obtained in the present experiment as relatively minor.

Suitable strains having been thus obtained, the next step will involve testing F_1, F_2 and backcross hybrids between the two extreme strains for the purposes of genetic analysis. Such a project has just been completed, using C57BR/a and A/Jax mice, and the results will be presented in a forthcoming paper.

TABLE I. Means and variances in activity-level shown by five inbred mouse strains in two test-situations

Strain	Test 1 N	Test 1 Mean	Test 1 Variance	Test 2 N	Test 2 Mean	Test 2 Variance
C57BR/a	40	532.2	22274.46	20	284.8	2480.04
C57BL/6	40	358.3	10887.14	20	181.0	416.16
AKR	40	185.0	27924.95	20	211.8	1014.01
BALB/c	40	37.4	6752.64	20	137.0	1239.04
A/Jax	40	11.4	1072.94	20	79.6	852.64



ACTIVITY LEVEL

Figure 21

The activity-level of five inbred mouse-strains as a function of time is illustrated graphically. It can be seen that the strains vary considerably in activity-level.

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